Intergeneric hybridisation in New Zealand Gnaphalieae (Compositae)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Botany

by

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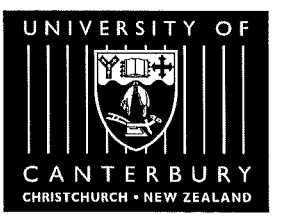

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Table Of Contents

List Of Figures

List Of Tables

List Of Plates

Abstract

The occurrence of natural intergeneric hybridisation among the New Zealand Gnaphalieae was investigated using a case study approach. Putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii* were collected from beside the Yeo Stream, Inland Kaikoura Range, Marlborough and putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* from Mount Hutt, Mount Hutt Range, Canterbury. Cytology, pollen stainability and experimental crosses provided evidence for reduced fertility in the putative hybrids. Field evidence and the morphology and leaf anatomy of the putative hybrids supported the hybridity hypotheses for the majority of the putative hybrids. A range of isolating mechanisms may restrict the frequency of these hybrids in the field. In particular, environmental factors (the availability of suitable habitats and natural disturbance) and pre-zygotic and post-zygotic barriers (embryo and/or endosperm abortion, hybrid fitness and hybrid fertility) were suggested to be important.

Cross-compatibility among indigenous Gnaphalieae and with related exotic Gnaphalieae was investigated through artificial crosses. Individual plants from six indigenous and five exotic species were preferentially selected as parents. The results provided evidence for the cross-compatibility of many indigenous Gnaphalieae, including species of *Anaphalioides*, *Euchiton*, *Ewartia*, *Helichrysum*, *Leucogenes* and *Raoulia*. A plant of *Euchiton audax* was cross-compatible with individual plants of *Ewartia planchonii* and *Gamochaeta spicata*. The results indicate species groups among the indigenous Gnaphalieae are less genetically distinct than morphology suggests. The partial fertility of some natural intergeneric hybrids suggests intergeneric gene exchange has a potential role in the future evolution of the group.

SECTION ONE

General Introduction

Chapter 1. Introduction

The Compositae are the largest family of eudicotyledons, comprising 17 tribes and approximately 23 000 species (Bremer, 1994 p. 13). The New Zealand everlasting daisies have traditionally been placed in the tribe Inuleae Cass. (e.g., Allan, 1961; Webb, 1988), but following a cladistic analysis Anderberg (1989) divided the Inuleae into three tribes (Gnaphalieae (Cass.) Lecoq & Juillet, Inuleae *sensu stricto* and Plucheeae (Cass. ex Dumort.) Anderb.) with the New Zealand taxa assigned to the Gnaphalieae. The Gnaphalieae are characterised by style arms with apically separated stigmatic lines, pollen grains with a two-layered sexine with an outer baculate and an inner perforated layer, and a base chromosome number of $x = 7$ (Anderberg, 1991). Most Gnaphalieae also have capitula which are discoid (contain one type of floret) or disciform (with two types of floret, the outer ones not ligulate), papery involucral bracts and entire leaves (Anderberg, 1994). The tribe has a worldwide distribution with centres of diversity in Australia, South Africa and South America. It is one of the largest tribes in the Compositae, comprising over 180 genera and 2000 species (Anderberg, 1994). An estimated 70-80 species in ten genera are indigenous to New Zealand (Ward and Breitwieser, 1998a).

1.1 The New Zealand Gnaphalieae

1.1.1 Overview of the genera

The endemic *Raoulia* Hook.f. is the largest genus of New Zealand Gnaphalieae, with 23 described species and eight entities that might warrant naming at species level (Ward, 1997). Anderberg (1991) divided the genus into *Raoulia sensu stricto* and *Psychrophyton* Beauverd, but Ward and Breitwieser (1998a) retained *Raoulia sensu lato* until the relationships of the constituent species are clarified. Thus, *Raoulia sensu* Allan (1961) is used in this thesis.

Euchiton Cass. contains about 20 species distributed in Asia and Australasia (see Ward & Breitwieser, 1998b), of which 14 species are native and at least six species endemic to New Zealand (Webb, 1988). These species were formerly placed in the genus *Gnaphalium* L. (e.g., Allan, 1961; Drury, 1972; Webb, 1988). Recent studies indicate *Euchiton* comprises distinct stoloniferous and non-stoloniferous species groups (Breitwieser and Ward, 1993; Ward, 1993; Breitwieser and Sampson, 1997a; Breitwieser and Sampson, 1997b; Breitwieser *et al.*, 1999).

Eight endemic species are retained in the genus *Helichrysum* Mill. corr. Pers. by Ward and Breitwieser (1998a), but as it is circumscribed by Hilliard and Burtt (1981) they must be excluded from this genus. The six 'whipcord' species are closely related, but the affinities of *H. filicaule* Hook.f. and *H. lanceolatum* are more obscure (Breitwieser and Ward, 1993; Ward, 1993b; Ward and Breitwieser, 1998a).

The genus *Anaphalioides* (Benth.) Kirp. was published by Kirpicznikov in 1950 but has only recently become more widely accepted (Merxmüller *et al.*, 1977; Anderberg, 1991; Glenny, 1997). As circumscribed by Glenny (1997), the genus contains five endemic species and two New Guinean species.

Leucogenes Beauverd is an endemic genus of four distinctive alpine species (Molloy, 1995), which are known colloquially as the New Zealand edelweiss.

Ewartia Beauverd (*sensu* Allan, 1961) comprises four Australian species and the New Zealand endemic *E. sinclairii* (Hook.f.) Cheeseman. Anderberg (1991) created the monotypic genus *Ewartiothamnus* Anderb. to accommodate *E. sinclairii*, but Ward and Breitwieser (1998a) retained *Ewartia sensu lato* pending resolution of the relationships of the constituent species.

Rachelia J.M.Ward & Breitw. is a recently described, endemic genus containing a single alpine species (*R. glaria* J.M.Ward & Breitw.) restricted to argillite screes in southern Marlborough and northern Canterbury. Its closest relatives among the indigenous New Zealand Gnaphalieae are uncertain (Ward *et al.*, 1997; Breitwieser *et al.*, 1999).

The Australasian genus *Ozothamnus* contains a single indigenous species complex, *O. leptophyllus* (G.Forst.) Breitw. & J.M.Ward, which was formerly included in *Cassinia* R.Br. (Breitwieser and Ward, 1997; Breitwieser and Ward, 1998). This species is only distantly related to other indigenous New Zealand Gnaphalieae and is hypothesised to have arrived in New Zealand independently (Breitwieser *et al.*, 1999).

All indigenous representatives of the genus *Pseudognaphalium* Kirp. are currently assigned to a single cosmopolitan species complex, *P. luteoalbum* (L.) Hilliard & B.L.Burtt (Webb, 1988). ITS sequences indicate this species is only distantly related to other indigenous New Zealand Gnaphalieae (Glenny and Wagstaff, 1997; Breitwieser *et al.*, 1999).

The genus *Craspedia* G.Forst. contains an uncertain number of endemic species. Its closest relatives are in the *Angianthus* group, which is centred in Australia, rather than other New Zealand Gnaphalieae (Merxmüller *et al.*, 1977; Anderberg, 1991; Breitwieser and Ward, 1993; Breitwieser *et al.*, 1999). Consequently, *Craspedia* was excluded from this thesis.

The endemic genus *Haastia* Hook.f. has previously been placed in the Inuleae (Merxmüller *et al.*, 1977; Webb, 1988), but it lacks the diagnostic characters of the Gnaphalieae (e.g., Breitwieser and Sampson, 1997a; Breitwieser and Sampson, 1997b) and is currently unassigned to any tribe (Bremer, 1994).

The Gnaphalieae are a problematic tribe regarding generic boundaries and relationships and the New Zealand taxa are no exception. Elucidation of generic limits and relationships has been the objective of recent studies of the New Zealand Gnaphalieae (Breitwieser, 1993; Ward, 1993a; Ward, 1993b; Jordan, 1995; Falvey, 1996; Glenny, 1997; Glenny and Wagstaff, 1997; Breitwieser and Sampson, 1997a; Breitwieser and Sampson, 1997b; Breitwieser and Ward, 1997; Wilton, 1997; Breitwieser *et al.*, 1999) and the present investigation of intergeneric hybridisation is a continuation of this work. Although the generic affinities of many species have been resolved, a number of problems remain, such as the correct position of *Ewartia sinclairii* and the indigenous *Helichrysum* species (Ward and Breitwieser, 1998a).

Traditionally, many New Zealand taxa were placed in two large cosmopolitan genera, *Gnaphalium* and *Helichrysum*, which were distinguished principally by the ratio of female to hermaphrodite florets in the capitula (Bentham, 1873a). The emphasis placed on this character, however, caused the separation of closely related taxa and the linkage of distantly related species (Hilliard and Burtt, 1981). As an example, Bentham (1873b) placed *Anaphalioides bellidioides* (G.Forst.) Glenny in *Helichrysum* but included *A. trinervis* (G.Forst.) Anderb. in *Gnaphalium.* Despite the discovery of new taxonomic characters (e.g., Drury and Watson, 1966; Drury, 1970; Hilliard and Burtt, 1981), subsequent delimitation of segregate genera has proved difficult because of the distribution of important character states in the tribe (Anderberg, 1994).

Raoulia exemplifies the taxonomic instability among the New Zealand Gnaphalieae. Hooker (1846) published the genus to accommodate the species *R. australis* Hook.f., but later noted the genus was characterised principally by its growth habit (Hooker, 1864). Subsequent authors (Bentham, 1873a; Kirk, 1899) also recognised the difficulty in distinguishing *Raoulia*

from *Gnaphalium* and *Helichrysum*. Beauverd (1910) split the genus into *Raoulia sensu stricto* and *Psychrophyton*, but subsequently relegated *Psychrophyton* to subgeneric level following examination of *R. petriensis* Kirk (Beauverd, 1912). Allan (1961) created the subgenus *Mistura* Allan to acknowledge the intermediate nature of *R. petriensis*. More recently, Ward (1993a, 1993b) and Breitwieser and Ward (1993) found *Raoulia* comprised two phenetically distinct groups and several species (*R. cinerea* Petrie, *R. petriensis*, the mat-forming species of subg. *Psychrophyton* and an undescribed taxon, *R.* sp. "M") of uncertain position. Ward (1993b) concluded the generic limits of *Raoulia* must be altered and suggested *R. cinerea* might warrant recognition as a monotypic genus. Anderberg (1991) resurrected *Psychrophyton* at generic level and placed *R. petriensis* in subg. *Raoulia*, but Ward and Breitwieser (1998a) retained *Raoulia sensu lato* until the relationships of the constituent species are fully clarified.

The generic limits of *Ewartia* are similarly uncertain. The single New Zealand endemic species, *E. sinclairii*, was transferred from *Helichrysum* by Cheeseman (1925), but it lacks the principal diagnostic character of *Ewartia* (i.e., subdioecy) (Ward, 1993b). Thus either the generic concept needs revising or *E. sinclairii* must be excluded from *Ewartia* (Breitwieser and Ward, 1993; Ward, 1993b). Anderberg (1991) concluded *Ewartia* was not monophyletic and created the monotypic genus *Ewartiothamnus* to accommodate *E. sinclairii*. Morphological, leaf anatomical and flavonoid evidence (Breitwieser and Ward, 1993; Ward, 1993b) and ITS sequences (Glenny and Wagstaff, 1997; Breitwieser *et al.*, 1999) suggest *E. sinclairii* is more closely related to other New Zealand Gnaphalieae than to Australian *Ewartia* species. Morphological, leaf anatomical, flavonoid and pollen data and ITS sequences suggest the Australian species are heterogeneous (Breitwieser and Ward, 1993; Ward, 1993b; Breitwieser and Sampson, 1997a; Breitwieser and Sampson, 1997b; Breitwieser *et al.*, 1999). Breitwieser *et al.* (1999) suggested a combined revision of Australian and New Zealand *Euchiton* and *Ewartia* is required to further elucidate the origins of the species.

The affinities of the endemic *Helichrysum* species still require resolving. Ward (1993b) suggested the endemic *Helichrysum* species might comprise at least three genera. Anderberg (1991) placed the whipcord species and *H. lanceolatum* in *Ozothamnus*, but ample evidence (e.g., Breitwieser and Ward, 1993; Ward, 1993b; Glenny and Wagstaff, 1997) suggests other New Zealand Gnaphalieae are their closest relatives. *Helichrysum filicaule* does not belong in the *Lawrencella* complex as Anderberg (1991) proposed (Ward and Breitwieser, 1998a) and although leaf anatomy and flavonoids suggest an affinity with *Anaphalioides* (Breitwieser and

Ward, 1993), *H. filicaule* is morphologically very distinct from *Anaphalioides* species (Glenny, 1997). *Helichrysum lanceolatum* is even more distinctive (e.g., Breitwieser and Ward, 1993; Ward, 1993b; Wilton, 1997).

Five endemic species were accepted in *Anaphalioides* by Glenny (1997). Previously, they have been placed in *Anaphalis*, *Gnaphalium*, *Helichrysum* and *Xeranthemum* L. (e.g., Forster, 1786; Bentham, 1873b; Allan, 1961; Webb, 1988). Other species and varieties have been described but are now reduced to synonymy.

The indigenous species of *Euchiton* and *Leucogenes* have had a comparatively stable taxonomic history. The *Euchiton* taxa have traditionally been placed in *Gnaphalium* section *Euchiton* (Cass.) DC. (e.g. Allan, 1961; Drury, 1972; Webb, 1988), but *Euchiton* is now accepted at generic level (Holub, 1974; Anderberg, 1991; Ward and Breitwieser, 1998a; Ward and Breitwieser, 1998b). *Leucogenes grandiceps* (Hook.f.) Beauverd and *L. leontopodium* (Hook.f.) Beauverd were formerly placed in *Gnaphalium* (Hooker, 1864) and *Helichrysum* (Hooker, 1853; Kirk, 1899) and transferred to the new genus *Leucogenes* by Beauverd (1910) where they have remained to the present day. Two additional species, *L. neglecta* Molloy and *L. tarahaoa* Molloy, have recently been described (Molloy, 1995).

It has been hypothesised that the indigenous species of *Anaphalioides*, *Ewartia*, *Helichrysum*, *Leucogenes*, *Rachelia* and *Raoulia* comprise a recently evolved group that has radiated within New Zealand from a single common ancestor (Glenny and Wagstaff, 1997; Ward, 1997). ITS sequences and morphology suggest the New Guinean *Anaphalioides* species are also members of this group (Glenny, 1997; Breitwieser *et al.*, 1999). *Euchiton*, *Craspedia*, *Ozothamnus leptophyllus* and *Pseudognaphalium luteoalbum* are hypothesised to have arrived in New Zealand independently (Breitwieser *et al.*, 1999).

1.1.2 Subtribal classification of the Gnaphalieae

Traditionally, the Inuleae were considered to form a well-defined 'natural' tribe within the subfamily Asteroideae. Bentham (1873b), in his tribe Inuleae (as Inuloideae), distinguished nine subtribes and placed the taxa with filiform florets and truncate styles (which includes all of the New Zealand taxa except *Craspedia*) in the subtribe Gnaphaliinae (as Gnaphalieae) (Table 1.1 p. 8). Bentham distinguished two generic groupings within the Gnaphaliinae: *Gnaphalium* and *Raoulia* were placed in the Eugnaphalieae, and *Helichrysum* and *Cassinia* in

Bentham (1873a)	Merxmüller *et al.* (1977)	Anderberg (1991)
Tribe Inuleae	**Tribe Inuleae**	**Tribe Gnaphalieae**
Subtribe Gnaphaliinae	**Subtribe Gnaphaliinae**	**Subtribe Loricariinae**
Eugnaphalieae group:	Anaphalis group:	*Psychrophyton* Beauverd
Anaphalis DC.	*Anaphalioides* (Benth.) Kirp.	
Antennaria Gaertn.	*Anaphalis* DC.	**Subtribe Cassiniinae**
Gnaphalium L.	*Antennaria* Gaertn.	Anaphalis group:
Leontopodium (Pers.) R.Br.	*Leontopodium* (Pers.) R.Br.	*Anaphalioides* (Benth.) Kirp.
Raoulia Hook.f.	Gnaphalium group:	*Anaphalis* DC.
Helichryseae group:	*Ewartia* Beauverd	*Antennaria* Gaertn.
Cassinia R.Br.	*Gnaphalium* L.	*Ewartia* Beauverd
Helichrysum Mill., corr. Pers.	*Leucogenes* Beauverd	*Ewartiothamnus* Anderb.
	Pseudognaphalium Kirp.	Cassinia group:
	Raoulia Hook.f.	*Cassinia* R.Br.
	Helichrysum group:	*Ozothamnus* R.Br.
	Cassinia R.Br.	*Raoulia* Hook.f.
	Helichrysum Mill., corr. Pers.	
		Subtribe Angianthinae
		Lawrencella complex:
		Anaphalioides bellidioides
		Helichrysum filicaule
		Subtribe Gnaphaliinae
		Gamochaeta Wedd.
		Gnaphalium group:
		Euchiton Cass.
		Gnaphalium L.
		Vellereophyton Hilliard & B.L.Burtt
		Helichrysum group:
		Helichrysum Mill., corr. Pers.
		Pseudognaphalium Kirp.
		Leontopodium group:
		Leontopodium (Pers.) R.Br.
		Leucogenes Beauverd

Table 1.1. Comparison of the classifications by Bentham (1873a), Merxmüller *et al.* (1977) and Anderberg (1991) of the gnaphalioid genera included in this thesis.

the Helichryseae. Only recently have major changes to Bentham's classification been proposed.

Merxmüller *et al.* (1977), utilising new cytological, palynological and phytochemical data, recognised only three subtribes within the Inuleae. All indigenous New Zealand gnaphalioid daisies, together with *Haastia pulvinaris* Hook.f., were distributed over three generic groupings in a more broadly defined subtribe Gnaphaliinae (Table 1.1). *Ewartia*, *Gnaphalium*, *H. pulvinaris*, *Leucogenes*, *Pseudognaphalium* and *Raoulia* were placed in the Gnaphalium group; *Anaphalioides* in the Anaphalis group; and *Cassinia* and *Helichrysum* in the Helichrysum group.

Following a cladistic analysis based on morphological data, Anderberg (1989) concluded the tribe Inuleae were paraphyletic and lacked any diagnostic characters. He split the Inuleae into three tribes: the Gnaphalieae, Inuleae *sensu stricto* and Plucheeae (Anderberg, 1991). The New Zealand Gnaphalieae were divided between four of the five subtribes he distinguished (Table 1.1).

A recent study of non-coding chloroplast DNA sequences by Bayer and Starr (1998) supported the paraphyly of the Inuleae *sensu lato* and segregation of the Gnaphalieae. The merit of Anderberg's subtribal classification is uncertain, however. Puttock (1994) highlighted deficiencies in Anderberg's methodology and reanalysed his data set, obtaining a different phylogeny and markedly shorter tree, with disintegration of the Cassiniinae being the most notable difference. *Anaphalioides*, *Ewartiothamnus* and *Ozothamnus* fell into an expanded Gnaphaliinae, but *Raoulia* was consistently placed in a group (the *'Lucilia* clade') outside the other subtribes. Wilson *et al.* (1992) also disagreed with Anderberg's subtribal classification of some Australian taxa.

Breitwieser and Ward (1993) did not accept Anderberg's subtribal classification of the New Zealand Gnaphalieae, as taxa that are morphologically and chemically similar (Breitwieser and Ward, 1993; Ward, 1993b) and phylogenetically related (Glenny and Wagstaff, 1997; Breitwieser *et al.*, 1999) are separated. Ward and Breitwieser (1998a) discussed shortcomings in Anderberg's data for the New Zealand Gnaphalieae and considered (p. 168), "... it is clearly inadvisable to accept his genera and subtribes uncritically". The confirmation of natural intergeneric hybrids between his subtribes (Jordan, 1995; Falvey, 1996) casts further doubt on Anderberg's subtribal classification of the New Zealand Gnaphalieae.

1.2 Definitions of hybrids

Harrison (1993) reviewed published definitions of 'hybrid' and 'hybridisation'. He defined (p. 5) hybrids as the result of "interbreeding of individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters". Such a definition is less satisfactory for individuals of mixed ancestry, such as backcross progeny and introgressants. Harrison also pointed out that a simpler definition of a hybrid as the "offspring of any pair of genetically distinct individuals" is acceptable in genetics and plant and animal breeding, where individuals possessing particular traits are selected as the parents, but in a natural population every outcross would thus yield a hybrid. A definition more meaningful for taxonomic studies is that of Stace (1975, 1986) who defined 'taxonomic hybrids' as the progeny of crosses between individuals of different taxa. Different kinds of hybrids reflecting their ancestry and the degree of evolutionary divergence can also be distinguished. Rieseberg and Ellstrand (1993), for example, recognised 'first generation (or F_1) hybrids', 'later generation hybrids' (including backcrosses) and 'hybrid species' (i.e., species of hybrid origin). McDade (1995) distinguished 'primary hybrids' (relatively genetically unmodified hybrids) and 'derived hybrids' (those that have undergone considerable evolutionary divergence). Multiple generations of hybridisation may result in the formation of local hybrid swarms or distinct hybrid zones. In the most extreme cases, regional clines or hybrid complexes may result, rendering the identification and classification of individuals difficult. A hybrid complex is "a species group, consisting of three or more original ancestral species and their hybrids or hybrid derivatives, in which natural hybridization has obscured the morphological discontinuities between the ancestral forms" (Grant, 1981 p. 273).

Application of the terms 'intergeneric' and 'interspecific' reflects the generally accepted taxonomic classification of the plants in question. Since opinions on the delimitation of species and genera can differ widely, especially in the Compositae, the status of the hybrids under investigation may conceivably change in future in line with taxonomic revisions. Hybrids resulting from hand pollinations are termed 'artificial' or 'experimental' hybrids; those arising in the field without the aid of man are termed 'natural'. Hybrids arising in cultivation but which do not result from hand pollinations are termed 'spontaneous'.

1.3 Brief synopsis of hybridisation in plants

Hybrids have been recorded among all major plant groups (Stace, 1975; Stace, 1989). In the British Isles about 730 interspecific hybrid combinations are well documented and Stace (1993) extrapolated this figure to hypothesise that 73 000 natural interspecific combinations

might occur worldwide. Artificial hybrids are much more common than natural hybrids and over 70 000 artificial hybrids have been synthesised in the Orchidaceae alone (Stace, 1993), indicating the vast potential for hybridisation in the plant kingdom. Hybrids between multiple species occur, an extreme example being a hybrid involving 13 *Salix* L. species raised by Nilsson (1954).

Natural hybridisation is not randomly distributed in vascular plants, but is concentrated in a small proportion of families and an even smaller number of genera (Stace, 1975). Ellstrand *et al.* (1996), in a survey of five biosystematic floras from the British Isles, Scandinavia, the United States and Hawaii, found the Compositae had a high incidence of hybridisation with four genera (*Aster* L., *Bidens* L., *Dubautia* Gaudich. and *Taraxacum* Weber) accounting for over 30 % of the 180 hybrids in the family. Genera with the highest frequency of natural hybrids were often perennial, outcrossing and with some means of clonal spread. However, such data should be interpreted with care; the intensity of study of hybrids in the respective floras may vary, differing opinions on species limits may influence hybrid frequency and the data was incomplete for some of the floras. In addition, the frequency of natural hybridisation varies with life history, pollination and breeding systems, environmental disturbance and genetic predisposition (Grant, 1981 p. 231) and survival of natural hybrids in the field is likely to vary.

Among plants intergeneric hybrids are much less frequent than intrageneric hybrids and interfamilial hybrids are unknown (Stace, 1986). Knobloch (1972) listed 2993 intergeneric hybrids distributed among 45 angiosperm families, about 90 % of which were found in the Orchidaceae and Gramineae, and artificial hybrids comprised over half of the total number (Stace, 1975). However, Stace (1975) expressed caution over accepting Knobloch's list in full. Some of the hybrids listed are erroneous (McComb, 1975) or now classified as intrageneric following changes in generic boundaries, e.g., *Arctotis* L. × *Venidium* Less. (Huxley *et al.*, 1992) and *Dubautia* × *Railliardia* Gaudich. (Carr, 1990). In addition, Knobloch did not distinguish between substantiated hybrids and those for which the parentage was only hypothesised.

Stace (1986, 1989) counted 17 natural intergeneric combinations in the British vascular flora and extrapolated this to estimate that 2 930 intergeneric combinations may occur naturally world-wide. Artificial intergeneric hybrids between taxa lacking the opportunity to cross naturally are much more frequent than natural hybrids, especially in the Orchidaceae and

Gramineae. Hunt and Hunt (1991) listed 554 intergeneric hybrid combinations in the Orchidaceae, but few natural intergeneric hybrids are known in this family (Stuessy, 1990 p. 200). In the Compositae intergeneric hybrids, both natural and artificial, are recorded in ten tribes (see Chapter 2). In general, natural intergeneric hybridisation is rare in families with specialised pollination systems, such as the Orchidaceae, and more common in families reliant on wind pollination (Stuessy, 1990 p. 201) or generalist pollinators.

Artificial hybrids involving three or more angiosperm genera have been synthesised, most notably in the Orchidaceae, in which hybrids involving six genera are recorded (Hunt and Hunt, 1991). However, no natural hybrids involving more than two genera are known (Stace, 1975). In the Compositae, experimental trigeneric hybrids involving species of *Dubautia*, *Madia* Molina and *Raillardiopsis* Rydb. have been raised (Carr *et al.*, 1996). Artificial intertribal crosses (e.g., between *Bromus* L. and *Festuca* L.) have been successful in the Gramineae (Stace, 1975) and in the Orchidaceae artificial crosses between members of different subfamilies may succeed (Solbrig, 1970).

1.4 Hybridisation in the New Zealand flora

Some authors have considered the New Zealand flora exhibits a high frequency of natural hybrids relative to other floras (Allan, 1931; Cockayne and Allan, 1934; Rattenbury, 1962). Cockayne and Allan (1934) listed over 400 hybrid combinations, but substantive data were largely lacking and some combinations were acknowledged to be speculative. Allan (1961) listed a wide range of putative hybrids among native gymnosperms and angiosperms, but was more circumspect in his assertions and discarded some of the combinations previously suggested. An extensive study of natural and artificial hybridisation in *Epilobium* L. supported the hypothesis that hybridisation has been important in the evolution of the genus within New Zealand (Raven and Raven, 1976). However, very few studies have substantiated the frequency or importance of natural hybridisation in the New Zealand flora (see Hair, 1966; Connor, 1985) and subsequent workers have been unable to confirm Cockayne and Allan's list of hybrids for certain genera, such as *Astelia* Banks & Sol. ex R.Br. and *Carex* L. (see Connor, 1985). Thus the statement by Hair (1966 p. 579) that, "The most that can be said about natural hybridisation in the New Zealand flora is that it is well attested in a few genera and is probable in others", is just as applicable today.

Excluding the indigenous Gnaphalieae, nine natural intergeneric hybrid combinations are recorded in the New Zealand flora (Table 1.2 p. 13). Several hybrids are now classified as

Intergeneric cross	Reference
Aciphylla J.R.Forst. & G.Forst. × *Anisotome* Hook.f.	Webb and Druce (1984)
Anisotome Hook.f. × *Gingidia* J.W.Dawson	Webb and Druce (1984)
Celmisia Cass. × *Olearia* Moench	Clarkson (1988); Heenan (1993)
Damnamenia Given × *Pleurophyllum* Hook.f.	Given (1973)
Carpobrotus N.E.Br. × *Disphyma* N.E.Br.	Chinnock (1972)
Elymus L. × *Stenostachys* Turcz.	Connor (1994)
Forstera L.f. × *Phyllachne* J.R.Forst. & G.Forst.	Mark (1995)
Ileostylus Tieghem × *Tupeia* Cham. & Schlecht.	Thompson (1949)
Kunzea Reichb. × *Leptospermum* J.R.Forst. & G.Forst.	Harris *et al.* (1992)

Table 1.2. Natural intergeneric crosses reported in the New Zealand flora (excluding the New Zealand Gnaphalieae). The list reflects currently accepted generic concepts. For combinations reported for the New Zealand Gnaphalieae, see Chapter 2.

intrageneric following redefinition of generic limits (e.g., *Gaultheria* Kalm ex L. × *Pernettya* Gaudich. and *Hymenanthera* R.Br. × *Melicytus* J.R.Forst. & G.Forst.). Eight natural intergeneric combinations in the New Zealand Gnaphalieae are reported in the literature (see Chapter 2). In addition, experimental hybrids between species of *Astelia* and *Collospermum* Skottsb. have been synthesised (Moore, 1980).

Natural hybrids between native and adventive species are recorded in *Acaena* L. (Macmillan, 1988), *Epilobium* (Raven and Raven, 1976) and between *Disphyma australe* (Sol.) J.M.Black and the adventive *Carpobrotus edulis* (L.) L.Bol. and *C. aequilaterus* (Haw.) N.E.Br. (Chinnock, 1972). Artificial hybrids between indigenous and exotic species have been synthesised in a number of genera, including *Cortaderia* Stapf (Connor, 1983), *Epilobium* (Raven and Raven, 1976), *Luzula* DC. (Nordenskiöld, 1971) and between Australian and New Zealand members of the *Senecio glaucophyllus* Cheeseman complex (Ornduff, 1962).

Natural intrageneric hybrids are recorded in a number of indigenous Compositae genera, but few examples have been investigated in any detail. Among Gnaphalieae, intrageneric hybrids are reported in *Raoulia* and among whipcord *Helichrysum* species (e.g., Allan, 1961; Williams, 1989; Dawson *et al.* 1993) and a range of putative intergeneric hybrids have been collected (see Chapter 2). Given (1984) produced a comprehensive list of putative natural hybrids between species of *Celmisia* subg. *Pelliculatae* section *Petiolatae* and species of other

subgenera, but substantive evidence was largely lacking. Free gene exchange is considered likely in only three out of the 25 combinations (Connor, 1985). Drury (1973) described the morphology and anatomy of intrageneric *Brachyglottis* J.R.Forst. & G.Forst., emend. B.Nordenstam hybrids. Most natural hybrids were classified as uncommon, but certain crosses were described as "high frequency", such as *B. elaeagnifolia* (Hook.f.) B.Nordenstam × *B. bidwillii* (Hook.f.) B.Nordenstam. Hybrids between herbaceous rosette-forming species and shrubby species occur, with natural hybrids between *B. cassinioides* (Hook.f.) B.Nordenstam and *B. haastii* (Hook.f.) B.Nordenstam reportedly common. Some spontaneous hybrids have arisen in cultivation. In *Leptinella* Cass., natural hybrids between several endemic species and artificial intersectional crosses are recorded (Lloyd, 1972; Lloyd, 1975).

1.5 The evolutionary significance of natural hybridisation

Classical writers tended to view hybrids as oddities or freaks (see Zirkle, 1935). Such a negative view of hybrids persists into modern times. For example, Hooker (1853 p. 15) believed hybrids are generally weak and usually barren and considered this to be the best evidence that new species could not evolve through hybridisation. Mayr (1963) considered, at least in animals, hybrids as "mistakes" that were rare and evolutionarily insignificant and, more recently (Mayr, 1992), that most plant hybrids are sterile or do not backcross with the parental species. Wagner (1970) considered hybrids to be "evolutionary noise" that have a negative impact on plant speciation and evolution. He believed most natural hybrids are sterile or ill-adapted and hinder rather than enhance speciation.

However, hybridisation is more usually considered to be a potentially important evolutionary process in plants. Hybridisation and subsequent changes in ploidy are believed to have been common in angiosperm evolution (Stebbins, 1950; Stace, 1975), but relatively few instances of homoploid hybrid speciation have been thoroughly documented (Rieseberg, 1997). Stace (1993) suggested hybridisation may be "part of the normal genetic pattern", rather than infrequent or inconsequential, in most multitypic genera of vascular plants. Ellstrand *et al.* (1996) considered hybridisation is unlikely to be a common adaptive mechanism in plants but is still probably important in plant evolution. They believed evolution following hybridisation is likely to be most frequent in groups in which hybridisation is most common and that hybridisation does not need to be widespread or adaptive to be of evolutionary importance. Hybridisation events resulting in infrequent, largely inviable or sterile hybrids could still give rise to new, evolutionarily stable lineages (Arnold, 1997 p. 24). Rieseberg (1997) suggested hybridisation might have a major role in speciation in small or peripheral populations.

Stebbins (1959, 1971) was another advocate of the evolutionary potential of hybridisation and considered its impact is strongly dependent on the environment in which it occurs.

As discussed earlier (p. 13), natural hybridisation is more frequent in some families and genera than others, but numerous factors other than genetic compatibility may influence this. For example, plant taxa adapted to specialist pollinators are less likely to hybridise than plants visited by generalist pollinators, which are more likely to carry heterospecific pollen. The rate of hybridisation is lower among self-fertile, inbreeding taxa (Rieseberg, 1997). Natural hybridisation is often associated with disturbed habitats (Anderson, 1949; Stebbins, 1959). Focke (1881) noted natural plant hybrids are more likely to occur when one of the parental species is rare or when the earliest or last flowers of one species coincides with peak flowering of the other parental species. Focke considered hybridisation is less likely if both parental species are abundant and have coincident flowering peaks. In Hawaiian *Bidens* and the Hawaiian silversword alliance (*Argyroxiphium* DC., *Dubautia* and *Wilkesia* A.Gray), natural hybridisation is limited principally by allopatry, but also differences in altitude, ecology, flowering phenology and pollinators between species (Ganders and Nagata, 1984; Baldwin, 1996).

1.5.1 Evolutionary consequences of hybridisation

Fisher (1965) distinguished eight patterns of reproductive interaction between species with overlapping distributions. The simplest situation is where there is geographic overlap with no interbreeding. Hybridisation may be evident as isolated hybrid individuals, hybrid swarms or hybrid zones, introgressive populations in which mixing of the parental genomes through genetic recombination results in blurring of taxonomic boundaries, or polymorphic populations comprising hybrid derivatives and in which none of the original parental forms remain. Hybrid derivatives may become genetically stabilised by selection, eventually creating comparatively homogeneous populations, or by allopolyploidy (i.e., genomic doubling following hybridisation). Thus the evolutionary impact of hybridisation can vary greatly.

Through recombination of the parental genomes, hybridisation promotes heterozygosity and rapidly increases genetic variation within populations. Segregation can result in a wide array of recombinant genotypes, many of which may be poorly adaptive, but some of which might have a selective advantage. However, hybridisation may result in disruption of coadapted

gene complexes, which is likely to be more severe in the F_2 generation and in some instances may increase developmental instability (Hochwender and Fritz, 1999).

Hybrids are expected to exhibit intermediacy in some characters and to display parental states in other characters. However, novel and extreme characters may arise in hybrids, especially in later generations (Rieseberg and Ellstrand, 1993; McDade, 1995), a phenomenon termed transgressive segregation (Tanksley, 1993). Many novel traits or new gene combinations may have low adaptive value, but some may give hybrids a selective advantage over the parental taxa. Hybrid dysgenesis, defined as the higher rates of chromosomal and genic mutation in hybrid populations, and transgressive segregation may be "the rule rather than the exception" in hybrid progeny (Rieseberg, 1997). Reports of novel traits in hybrids are not uncommon. Grant (1956) reported obtaining individuals with the number of floral parts exceeding or fewer than that of the parental taxa in amphiploid *Gilia* Ruiz & Pav. hybrids. Vassilevska-Ivanova *et al.* (1996) reported the occurrence of a novel character (tubular ray florets) in F_4 hybrids between *Helianthus annuus* L. and *Verbesina helianthoides* Michx. First-generation hybrids between *Tripleurospermum tetragonaspermum* (F.Schmidt) Pobed. and *Matricaria recutita* L., which both lack receptacular scales, unexpectedly possessed this character (Mitsuoka and Ehrendorfer, 1972). The expression and function of multiple alleles may vary in polyploids, allowing the products of alleles to evolve new roles (McDade, 1995). In addition, hybrid zones may contain an increased frequency of rare alleles, possibly because of higher mutation rates, intragenic recombination or relaxed selection (see Barton and Hewitt, 1985).

Introgression, which is the exchange of genes between two taxa by hybridisation and backcrossing (Anderson and Hubricht, 1938), may have a number of evolutionary consequences: for example, the dilution or loss of alleles of one taxon, severe outbreeding depression or elimination of locally adapted populations and rare taxa (Rieseberg, 1991b). However, the introduction of new genes through hybridisation may be beneficial for a population, as the new alleles may have a selective advantage or enhance the adaptability of the population. An example of introgression involving species of differing ploidy levels is *Senecio vulgaris* var. *hibernicus* Syme, which unlike the typical variety of *S. vulgaris* L. possesses ligulate florets and has evolved through introgressive hybridisation between *S. vulgaris* and the radiate *S. squalidus* L. (Abbott and Lowe, 1996). Introgression may also occur between members of closely related genera. For example, *Purshia glandulosa* Curran

was hypothesised to have evolved through introgression between *P. tridentata* DC. and *Cowania stansburiana* Torr. (Strutz and Thomas, 1964).

Hybridisation may lead to the 'genetic swamping' of rare taxa or small populations (Rieseberg and Ellstrand, 1993). Interbreeding between native and introduced species may result in the production of hybrids with invasive tendencies (Hollingsworth *et al.*, 1999) or the evolution of new taxa (Abbott, 1992) and may enhance displacement of the native species by the introduced species (Huxel, 1999). However, the impact of introgression in such cases will depend on the rate of gene exchange, the selective advantage of the new genotypes and hybrid fertility (e.g., see Huxel, 1999).

Hybridisation may also lead to the reinforcement of reproductive barriers between taxa (see Abbott, 1992; Rieseberg and Wendel, 1993). The model of reinforcement, originally described by Dobzhansky (1940), proposes that selection against hybridisation occurs in zones of contact between previously allopatric taxa, thus ensuring reproductive isolation between the two taxa. Such selective pressure may result from a number of factors. Interspecific pollen transfer may cause pollen wastage and stigma clogging, thus reducing the reproductive fitness of individuals (Pleasants, 1983; Rathcke, 1983). Hybrid offspring may also be considered a waste of resources, especially if they are weak or poorly adapted, and they may compete for habitat space, resources and pollinators with pure-bred individuals. Interspecific pollen transfer might have been one factor contributing to selection for staggered flowering times among several riverbed-inhabiting *Raoulia* species (see Wilton, 1997).

Estimates of hybrid fitness vary with genotype, habitat and with the measure used (see Arnold and Hodges, 1995). For example, the fitness of different Louisiana iris hybrid genotypes varies with habitat (Cruzan and Arnold, 1993; Emms and Arnold, 1997). Transplant experiments indicate hybrids between two subspecies of *Artemisia tridentata* are better adapted to the distinct soils in a hybrid zone than the parental taxa (Wang *et al.*, 1997; Wang *et al.*, 1998). Hybrid derivatives might occupy a novel or intermediate habitat (Cruzan and Arnold, 1993) or they might be confined to disturbed habitats (Stebbins, 1969). In some instances, hybrid genotypes have become more widespread than the parental species or have invasive tendencies (Stace, 1975; Raybould *et al.*, 1991; Hollingsworth *et al.*, 1999).

Heterosis, which is associated with the proportion of heterozygous loci in an individual, is mainly associated with the F_1 generation (Stace, 1975). Heterotic effects can be positive or

negative and affect a variety of structures and processes, such as vigour, organ size, and reproductive capacity. Hybrid vigour is a common positive manifestation, but hybrids may also be weak or chlorotic and die before maturity. Hybrid inviability, defined by Grant (1981 p. 115) as consitutional weaknesses that block gene exchange between species in the vegetative phase of the F_1 generation, is recorded in some Compositae hybrids. For example, Hollingshead (1930) obtained evidence for a lethal gene in *Crepis tectorum* L. that is detrimental only in hybrid combinations and only with some species.

Hybridisation between two taxa might be extremely rare, but the hybrid derivatives might have a competitive advantage relative to the parental taxa in a particular habitat (e.g., Cruzan and Arnold, 1994), or exploit new habitats and become more widespread than the parental taxa, as in hybrids between *Symphytum asperum* Lepech. and *S. officinale* L. (Stace, 1975). Stace (1993) suggested the most evolutionarily important hybridisation events may have been rare occurrences in which highly sterile hybrids resulted.

Sterility in the F_1 generation is not necessarily an evolutionary barrier. Fertility can be restored by allopolyploidy provided there is no chromosome pairing between the two parental genomes. The resulting allopolyploids are often reproductively isolated from the parental taxa. The production of euploid gametes by hybrids is another mechanism by which interspecific or intergeneric gene exchange may occur (Stace, 1993). Even highly sterile hybrids can occasionally produce fertile gametes and viable offspring, as in *Festuca rubra* L. × *Vulpia fasciculata* (Forsskål) Fritsch (Stace, 1993). Some sterile hybrids reproduce apomictically, as in the Compositae genera *Antennaria*, *Crepis*, *Hieracium* and *Taraxacum* (Grant, 1981 pp. 423–424). Absolutely sterile hybrids might become widespread by vigorous vegetative spread, as in some *Mentha* L. hybrids (Stace, 1975). *Spartina anglica* C.E.Hubbard is an allopolyploid derived from a sterile F_1 hybrid that has become more widespread in Britain than the parental species (Raybould *et al.*, 1991). Although the F_1 generation might be of low fertility, the fertility might increase in backcrosses and subsequent generations (e.g., Carr, 1995). Even intergeneric hybrids are often partially fertile, as indicated by the existence of multi-generic and later-generation hybrids (e.g., Hunt and Hunt, 1991; Carr *et al.*, 1996; Vassilevska-Ivanova *et al.*, 1996). However, highly fertile hybrids may still be reproductively isolated owing to various pre-zygotic and post-zygotic barriers, such as reproductive phenology, ecological separation and allopolyploidy (see Grant, 1981 pp. 114–116).

1.5.2 Plant taxa of hybrid origin

'Stabilisation' of hybrids results in true-breeding populations or individuals that might give rise to new taxa. Hybrid genomes can become stabilised after only a few generations (Rieseberg, 1997). Grant (1981 p. 243) listed six potential mechanisms by which hybrids may become stabilised: asexual reproduction; translocation heterozygosity; unbalanced polyploidy; amphidiploidy; recombinational speciation; and hybrid speciation. Sexual reproduction is limited or absent in the first three mechanisms and thus the latter three mechanisms are more important for speciation. Stabilisation of hybrids and restoration of their fertility by allopolyploidy may be an important mode of plant evolution (Stebbins, 1971). A number of criteria must be fulfilled for speciation via hybridisation, such as ecological divergence, a selective advantage for hybrids in this habitat and rapid chromosomal evolution (Rieseberg, 1997). A theoretical study by McCarthy *et al.* (1995) found that hybrid speciation is more rapid if the plant is an inbreeder. However, the eight documented cases of homoploid hybrid speciation accepted by Rieseberg (1997) all involve outbreeders. Rieseberg suggested lower levels of natural hybridisation among selfing plants may account for the discrepancy. Speciation involving a ploidy change might be more common as it confers a high degree of reproductive isolation from the parental species more rapidly than without a change in chromosome number (Brochmann *et al.*, 2000).

The genus *Helianthus* contains the best documented examples of hybrid speciation in the Compositae. Three annual species (*H. anomalus* Blake, *H. deserticola* Heiser and *H. paradoxus* Heiser) are believed to have evolved independently following hybridisation between *H. annuus* and *H. petiolaris* subsp. *fallax* Heiser, based on isozyme, nuclear ribosomal DNA and chloroplast DNA evidence (Rieseberg *et al.*, 1990; Rieseberg, 1991a). The three taxa are geographically and ecologically separated from the parental species. Although molecular markers suggest *H. paradoxus* is of recent hybrid origin, morphology, biochemistry and ecological distribution do not suggest intermediacy between the putative parental taxa. Rieseberg (1991b) suggested these might be under strong selective pressure, whereas the molecular markers are more neutral. He also suggested hybridisation might have provided new variation or recombination that enabled *H. paradoxus* to adapt to a distinct habitat. Chromosomal rearrangements appear to be important in the reproductive isolation of *H. anomalus*. The three species possess less genetic diversity than either parental species, suggesting a small number of individuals were involved in their origin (Rieseberg, 1997). Rieseberg (1991a) also hypothesised two other hybridisation events in *Helianthus* from which *H. bolanderi* A.Gray, *H. exilis* A.Gray and *H. debilis* subsp. *silvestris* Heiser have evolved.

Stephanomeria diegensis is believed to be derived from *S. exigua* Nutt. and *S. virgata* Benth. (Gallez and Gottlieb, 1982). Of 20 isozyme loci analysed, *S. diegensis* had only one rare allele unique from those of the parental taxa. Natural hybrids between the parental species occur in southern California and F_1 hybrids have low pollen viability, while in F_1 hybrids between *S. diegensis* and the other two species only 1-2 % of the pollen was viable. Rapid chromosomal evolution may have been important in the divergence of *S. diegensis* (Gottlieb, 1972).

A homoploid hybrid origin is also hypothesised for *Encelia virginensis* A.Nelson (Allan *et al.*, 1997) and *Senecio squalidus* L. (Abbott *et al.*, 2000). Three additional British *Senecio* L. taxa are believed to have evolved by allopolyploidy from hybrids between indigenous and introduced species (see Abbott and Lowe, 1996). Hybridisation between the adventive *Senecio squalidus* and the native *S. vulgaris* has given rise to the allohexaploid *S. cambrensis* (Ashton and Abbott, 1992), *S. vulgaris* var. *hibernicus* (Abbott *et al.*, 1992) and an unnamed allotetraploid stabilised introgressant (Irwin and Abbott, 1992). The North American species *Tragopogon miscellus* and *T. mirus* are other examples of allopolyploid species of recent, multiple origins in the Compositae (Soltis *et al.*, 1995). Other species in the Compositae hypothesised to be of hybrid origin include *Argyranthemum sundingii* Borgen (Brochmann *et al.*, 2000) and ancient hybridisation might have contributed to the genetic constitution of at least five species of *Dubautia* (Baldwin, 1998). Glenny (1997) speculated that *Anaphalioides hookeri* (Allan) Anderb. (2n = 56) may have evolved from hybrids between *A. bellidioides* and *A. trinervis* (both 2n = 28). Hybridisation might also have been important in the divergence of the subtribe Helianthinae (Rieseberg *et al.*, 1993; Schilling and Panero, 1996) and the origin of the Hawaiian silversword alliance (Barrier *et al.*, 1999).

In the New Zealand flora, strong substantive evidence for the hybrid origin of an indigenous taxon has yet to be published. However, hybridisation is postulated to have been evolutionarily important in, for example, the radiation of *Epilobium* in New Zealand (Raven and Raven, 1976) and the origin of *Podocarpus totara* var. *waihoensis* Wardle (Wardle, 1972), *Ranunculus haastii* subsp. *piliferus* F.J.F.Fisher and *R. nivicola* Hook.f. (Fisher, 1965).

1.6 Taxonomic implications of hybridisation

Circumscription of taxa at the genus and lower taxonomic ranks can be seriously hindered by genetic exchange between taxa. Through hybrid swarms, introgression and clines, hybridisation can result in continuous variation, blurring taxonomic boundaries and rendering the classification of individuals extremely difficult. A number of solutions are available to

taxonomists but the resulting taxonomic boundaries may be arbitrary or morphological differences may be obscured or overemphasised. In such cases the degree of morphological differentiation of the species and the extent of hybridisation must be considered (Davis, 1978).

The extent of convergence resulting from natural hybridisation may lead to differing taxonomic interpretations. If most individuals can be confidently assigned to distinct taxa, the intermediate forms could be treated as hybrids. However, if the intermediates are common or outnumber the parental taxa, or if introgressive or clinal variation exists, treatment of all individuals as conspecific may be the best solution (Fisher, 1965). Identification of hybrids can be difficult when highly variable taxa are involved. In Hawaiian *Bidens* some hybrids have been erroneously described as species and some species considered to be hybrids (Ganders and Nagata, 1984). A number of putative intergeneric hybrids in the New Zealand Gnaphalieae have been described as species owing to the distinct morphology of the parental taxa and the putative hybrids (see Table 2.1 p. 36).

Classifications often attempt to reflect evolutionary relationships, but there are problems with fitting multidimensional phylogenetic data into a two-dimensional hierarchical classification system (Stuessy, 1990). Many taxonomists seek to delimit strictly monophyletic groups (in the cladistic sense), which contain a common ancestor and *all* of its descendents. However, taxa of hybrid origin have two (or, rarely, more) ancestors. Various authors (e.g., Brummitt, 1997) have argued the difficulty of achieving monophyletic groups within the existing hierarchical classification system. Cronquist (1985) believed supraspecific plant taxa are not typically monophyletic and noted the difficulty of delimiting monophyletic groups in the Compositae. Because a classification system comprised solely of monophyletic groups is unable to cope with reticulate relationships (i.e., taxa of hybrid origin), Sosef (1997) argued a monophyletic model is unsuitable for the classification of nature.

1.6.1 The taxonomic value of hybridity and cytogenetic data

Cytogenetics evaluates the degree of relationship between taxa using data such as chromosome pairing, cross-compatibility and hybrid fertility (Stuessy, 1990 p. 317). Cytogenetic data provide valuable evidence for genetic affinity and evolutionary relationship between taxa. Consequently, such data have been utilised in a variety of plant families to aid the determination of taxonomic boundaries and phylogenetic relationships (e.g., Kruckeberg, 1962; Powell, 1985; Chuang and Heckard, 1991; Orgaard, 1994; Assadi and Runemark,

1995). Numerous authors have attempted to define taxonomic categories using cytogenetic and cross-compatibility criteria (see Stuessy, 1990). However, numerous ecologically distinct forms might exist within species defined by morphological or breeding criteria (e.g., Turesson, 1922). In addition, an unsuccessful cross or absence of natural hybrids is not proof of a distant relationship, as numerous internal and external factors may be involved (see Grant, 1981 pp. 111–117). In addition, some cytogenetic events, such as chromosome pairing, are genetically regulated in some plants and thus crossing data should be interpreted with caution (Solbrig, 1968).

Some authors (e.g., Donoghue, 1985; Funk, 1985b) consider crossing data are plesiomorphic (i.e., not unique or novel), as the ability to hybridise is an ancestral state, and are of no use in phylogenetic analyses. However, cytogenetic data are interactive rather than comparative and are more comparable to coefficients of association than synapomorphic data and instead should be used to test phylogenetic hypotheses based on comparative data (Stuessy, 1990 p. 201). Stace (1986) evaluated the merits of hybridity data for assessing taxonomic relationships and concluded they are of no value as an "absolute" criterion for taxa because there is no consistent correlation with morphological variation. Stace concluded such data are only of comparative value and recommended they should be treated on a par with other kinds of evidence.

Allan (1937, 1940b) was an advocate of the taxonomic value of studying wild hybrids and obtaining field evidence for hybridity. He also noted (Allan, 1940b) the taxonomic importance of intergeneric hybrids in the evidence of relationships they provide. However, the cytogenetic study by Mitsuoka and Ehrendorfer (1972) highlights how hybridisation data must be considered simultaneously with other evidence, and how taxonomic decisions should be based on the overall available evidence. Their results suggested *Chamaemelum* Mill. and *Anthemis* L. have a strong genetic affinity, but embryological, morphological and phytochemical evidence suggests *Chamaemelum* is more closely related to *Matricaria* L. In contrast, the two subgenera in *Anthemis* (subg. *Anthemis* and subg. *Cota* (J.Gay ex Guss.) Rouy) were less genetically compatible and, given differences in fruit anatomy and biochemistry between the two subgenera, there may be some justification for recognising *Cota* J.Gay ex Guss. at generic level. The potential lack of congruence between morphology and cross-compatibility is also exemplified by the Hawaiian silversword alliance, in which adaptive radiation has resulted in high morphological and ecological diversity without accompanying reproductive isolation (Baldwin, 1998).

Because morphology and interfertility are not consistently correlated, the upper taxonomic limit of hybridisation varies among plant families. Hybrids between intraspecific taxa may be sterile or impossible because of ploidy differences, differing chromosome numbers, specific genes, minor chromosomal arrangements or cytoplasmic incompatibility, but partially fertile intergeneric hybrids occur in some families and intersubtribal hybrids are recorded in the Gramineae (Stace, 1975).

Opinions differ on the taxonomic level at which hybridity and cytogenetic data are of value. Some authors have advocated species should be reproductively isolated (e.g., Mayr, 1942). Cain (1959) considered cross-compatibility data were useful only at the species level, implying that genera should not hybridise. Some authors suggest the distinctness of genera that hybridise is doubtful (Rollins, 1953) or that intergeneric hybrids should be highly sterile (Powell, 1985). Other authors (e.g., Kruckeberg, 1962) recognise that cross-compatibility alone is insufficient evidence for uniting genera and such data should agree with other types of evidence for such an argument to be valid. Powell (1985) considered that artificial crosses are a valuable supplement to other types of evidence when investigating generic limits in the Compositae, and that crossing data are more useful at the generic level than at specific or intraspecific levels. If a genus is not interfertile with any other genus, cross-compatibility data can still be useful to test intrageneric relationships (Rollins, 1953). Baker (1958) considered only hybrid fertility should be taxonomically important, while Löve (1963) considered natural hybridisation was of greater importance for classifications than artificial hybridisation.

The classification of species based on the degree of interfertility in experimental crosses has been advocated in the past (e.g., Clausen *et al.*, 1945), but logistics often preclude such experimentation being feasible, especially when large numbers of species are involved, and such criteria have not been widely accepted. Interspecific cross-compatibility is notably high in *Quercus* L. and *Salix* but is not a critical criterion for delimiting species in these genera (Davis, 1978). *Crepis eritreensis* Babcock and *C. thompsonii* Babcock were separated from *C. foetida* L. on morphological and physiological evidence, even though experimental hybrids between the taxa have relatively high fertility (Babcock, 1940). Stace (1975) argued that the classification of two interfertile taxa should be in relation to the interfertility of other closely related taxa within the same genus. For example, if only two species within a genus are interfertile, it may be best to reduce them to subspecies. In addition, giving undue weight to cross-compatibility and hybrid fertility may result in unnatural or inconsistent classifications (Babcock, 1940). Stuessy (1990 p. 201) considered all possible crosses ideally should be

attempted in order to understand the full significance of cross-compatibility results. Stuessy also considered it is important to estimate hybrid fertility, as high fertility is indicative of a close genetic affinity between the parental species.

1.6.2 Reproductive isolation and species concepts

The species has traditionally been considered the fundamental category in systematics and numerous species concepts have been published (see Stuessy, 1990 p. 161–181). A 'species' encompasses "groups of individuals (populations) recognised by any means", but the term also refers to the taxonomic level or rank at which the groups are placed (Davis and Heywood, 1963 p. 90). In plants 'species' (as a taxonomic category) is used for different types of populations with different life forms or mating systems (see Grant, 1981 pp. 3–41), hence different criteria may be required to define species in different genera or families. Evidence obtained from a variety of fields, such as morphology, anatomy, phytochemistry, cytology, breeding systems, ecology and geographical distribution, can be used to delimit species. Reproductive isolation and cytogenetic data have important implications for a number of species concepts.

Mayr (1942 p. 120) defined the 'biological species' as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups". Mayr (1963) later modified this definition, arguing fertile hybrids are an indication that the hybridising forms are members of the same species and that most interspecific hybrids are completely sterile. Mayr (1982 p. 273) further modified this definition to include ecological considerations: "a reproductive community of populations (reproductively isolated from others) that occupies a specific niche in nature". However, the difficulty of defining 'niche' means this definition has not received general approval. Other authors, such as Ehrlich (1964), reject a species concept based principally on reproductive factors. In practice the biological species concept has various weaknesses: for example, the difficulty of measuring the degree of reproductive isolation of all populations; cross-compatibility and morphological similarity are not always correlated; various barriers may prevent taxa hybridising in the field even though artificial crosses may be successful; some individuals may be reproductively isolated but morphologically indistinct, such as some polyploids and plants that reproduce asexually; and a hybrid's fertility may be anywhere on a continuum between completely sterile and fully fertile (Stace, 1975). As an example, all Hawaiian species of *Bidens* appear to be interfertile and so if cross-compatibility were to be used as a criterion for delimiting species, only a single species exhibiting greater morphological variation than the remainder of

the genus would be recognised (Ganders and Nagata, 1984). Consequently, these authors recognised species on the basis of morphological and ecogeographical evidence.

Reproductive isolation is also a component of the 'genetic species' concept, but in this case the degree of genetic difference between populations is primarily used to define species (Stuessy, 1990). The major shortcoming of this species concept is the difficulty of determining genetic distances.

The 'morphological species' concept is the most frequently used (Stuessy, 1990), as in many instances reproductive behaviour is insufficiently known to enable the biological species concept to be applied. In this case the circumscription of species is based on morphological discontinuities. The lack of general correlation between morphology and interfertility (Stace, 1986) is irrelevant with this species concept, thus hybridisation between morphologically distinct taxa is allowable. However, this might make it difficult to delimit hybridising taxa, even though the extreme forms are morphologically distinct, because of introgression or continuous variation. Consequently, decisions on where to draw taxonomic boundaries and the number of taxa recognised can be rather arbitrary.

Ancestry is an important component of 'phylogenetic species' concepts, by which species are diagnosed by possessing unique derived characters or unique combinations of characters (e.g., Nixon and Wheeler, 1990; Olmstead, 1995). Hybridisation has important implications for the phylogenetic species, as species should be monophyletic (i.e., all member populations are descended from a common ancestor). However, many plant species may be paraphyletic (Rieseberg and Brouillet, 1994) and populations of hybrid origin have at least two ancestors. Thus, depending on the extent of introgression and selection, it may prove difficult to classify some populations using a phylogenetic species concept. In addition, minor morphological differences or character states under simple genetic control may be overemphasised in this species concept.

Some authors have distinguished alternative categories of taxa reflecting reproductive limits and the degree of interfertility (see Stace, 1975 pp. 7-8). Stuessy (1990) used the collective term 'biosystematic species concepts' for these categories, which placed greater emphasis on reproductive affinity than morphological discontinuities. None have been widely adopted, perhaps because of the greater complexity of the nomenclature and the difficulty of classifying individuals without performing extensive artificial crosses.

More recently, de Queiroz (1998) advocated a 'general lineage concept of species', in which different species concepts emphasise different aspects of the evolutionary processes or stages of "lineage individuation".

1.6.3 Generic concepts in the Compositae

Historically, delimitation of genera has proved difficult in the Compositae. Many recognisable species groups are interconnected by intermediate species or shared characters, as in the Gnaphalieae (Anderberg, 1994). Discrimination of genera that are both monophyletic (i.e., contain *all* of the descendents of a common ancestor) and defined by synapomorphies (i.e., shared derived character states) can be difficult in the family (Funk, 1985a). Cronquist (1985 p. 7), discussing the problem of delimiting "conceptually useful" genera in the Compositae, wrote:

> "A determined search for well defined and well characterized genera in the Compositae all too often turns into a pursuit of a will-o-the-wisp, leading into a swamp from which one can exit only by abandoning common sense and following one of three trails, all leading to the River Styx: hopelessly large and meaningless genera, hopelessly small and trivial genera, or genera that provide only for species of a limited region and do not apply elsewhere."

Intergeneric hybrids (both natural and artificial) have been reported in ten tribes (see Chapter 2). In some instances, their existence has been a consequence of misplaced generic boundaries. However, many genera that are cross-compatible with other genera are recognised in the Compositae.

Opinions vary on the implications of cross-compatible genera for classifications. Rollins (1953) believed the existence of intergeneric hybrids casts doubt on the distinctness of the genera concerned. Löve (1963) considered generic boundaries are suspect if natural intergeneric hybrids occur frequently, but not necessarily if only artificial intergeneric hybrids can be produced. The fertility of intergeneric hybrids has also been deemed important (Powell, 1985). In contrast, Nesom (1994), who recognised numerous segregate genera in the Astereae, considered the existence of intergeneric hybrids in this tribe is not strong reason for broadening generic concepts to accommodate both parental species in the same genus. Similarly, *Argyroxiphium*, *Dubautia* and *Wilkesia* are recognised as distinct genera despite the occurrence of partially fertile natural and artificial intergeneric hybrids (Carr and Kyhos, 1986; Carr, 1990; Kyhos *et al.*, 1990; Carr, 1995; Carr *et al.*, 1996). Recently, Baldwin (1999)

reinstated or segregated seven genera in the Heliantheae, despite the cross-compatibility of some genera, in order to produce a classification that is better reflective of phylogenetic relationships.

The problem of delimiting cross-compatible genera in the Compositae is illustrated by the comments of Hartman and Lane (1991 p. 327) on the generic limits of *Haplopappus* and related genera:

> "If all were joined to Hall's (1928) *Haplopappus* (in which case the genus name should be *Xanthocephalum* based on priority) as has been suggested by some workers, then it would follow that yet other genera of Astereae should also be united with it. Ultimately, the collapse of most if not all of the tribe into one or a very few genera would result. Such a situation would obscure rather than clarify the phylogeny of the Astereae; for this reason we continue to recognise distinct genera, even though their members may occasionally hybridize."

The difficulty of delimiting genera in the Gnaphalieae is well illustrated by the circumscription of *Gnaphalium* and *Helichrysum.* Different (and often conflicting) concepts of these genera were used by Linnaeus and contemporary taxonomists (reviewed by Hilliard and Burtt, 1981). As circumscribed by Bentham (1873b), the two genera were cosmopolitan and differentiated primarily by the female to hermaphrodite floret ratio, but some species proved difficult to classify and some closely related species were separated (Hilliard and Burtt, 1981). Kuntze (1891, 1898) united many genera in Bentham's subtribe Gnaphaliineae; his concept of *Gnaphalium*, for example, included *Achyrocline* (Less.) DC., *Anaphalis*, *Antennaria*, *Facelis* Cass., *Helichrysum* and *Lucilia* Cass. In contrast, subsequent authors sought to split *Gnaphalium* and *Helichrysum* (*sensu* Bentham) into smaller, more natural groupings (e.g., Hilliard and Burtt, 1981) and a cladistic analysis of morphological data by Anderberg (1991) suggested *Gnaphalium sensu lato* and *Helichrysum sensu lato* are polyphyletic. Numerous segregate genera are now recognised, some of which are believed to have little affinity with *Gnaphalium sensu stricto* and *Helichrysum sensu stricto* (see Anderberg, 1991), indicating the artificiality of earlier generic concepts.

Species groups within *Gnaphalium* have been differentiated by a number of characters, such as the type of achenial hair and degree of fusion of the pappus (e.g., Drury, 1970; Drury, 1971). Some of these groups have been recognised at generic rank, e.g., *Euchiton* Cass. (Cassini, 1830) and *Gamochaeta* Wedd. (Weddell, 1856), but only recently have these genera

gained wider acceptance (e.g., Holub, 1974; Anderberg, 1991; Anderberg, 1994; Ward and Breitwieser, 1998b). Hilliard and Burtt (1981) utilised new morphological characters to establish five new genera and redefine or reaffirm the status of other segregate genera. Anderberg (1991) concluded *Gnaphalium sensu lato* was polyphyletic and recognised a number of segregate genera. In Anderberg's (1991) concept, *Gnaphalium sensu stricto* is still a cosmopolitan genus but is restricted to about 50 species.

As circumscribed by Anderberg (1991, 1994), *Helichrysum* is a mainly southern African genus but extends to Europe and Asia. All Australian and New Zealand taxa previously placed in this genus are misplaced in *Helichrysum sensu stricto* (Anderberg, 1991; Ward and Breitwieser, 1998a). Some species have been reclassified in *Anaphalioides*, *Anemocarpa* Paul G. Wilson, *Argentipallium* Paul G. Wilson, *Bracteantha* Anderb. & Haegi, *Lawrencella* Lindl., *Ozothamnus* R.Br. and *Schoenia* Steetz, but *Helichrysum sensu* Anderberg may still be paraphyletic or polyphyletic (Anderberg, 1991). Other generic boundaries, such as between *Achyrocline*, *Helichrysum* and *Pseudognaphalium*, also require further investigation (Anderberg, 1991).

In the Compositae, morphological criteria alone do not always satisfactorily discriminate natural genera or species groups, so often other types of evidence are required to help delimit genera. Hybridity and cytogenetic data have helped determine the correct generic placement of species (e.g., Heiser, 1963; Arnold and Jackson, 1978; Stucky, 1978), assess the distinctness of genera (e.g., Harms, 1965; Olorode and Torres, 1970; Powell, 1972), and evaluate generic relationships (e.g., Mitsuoka and Ehrendorfer, 1972; Powell, 1972; Powell, 1978; Powell, 1985; Kyhos *et al.*, 1990). Powell (1985) suggested artificial hybridisation may be of widespread use in the Compositae to help solve generic problems and identify natural species groups.

A desire for objectivity and repeatability in classifications resulted in the development of phenetics and cladistics, but these methods may suggest quite different species groups. Phenetic algorithms seek to discriminate taxa free from bias and with all characters weighted equally (Sneath and Sokal, 1973). However, phenetics does not attempt to represent evolutionary relationships and trivial characters may assume undue importance (Clayton, 1983). In cladistics, emphasis is placed on monophyly of groups. Cladists consider a genus should contain *all* of the descendents of a common ancestor and be based on synapomorphies, i.e. shared derived character states (e.g., Funk, 1985a; Nelson, 1989). However, Cronquist

(1985) considered the pursuit of absolute monophyly was more destructive than helpful and concluded acceptance of ill-defined genera in the Compositae is necessary in order to have "conceptually useful" genera. Because taxa of hybrid origin are derived from more than one ancestral lineage, including monophyly as a criterion for generic delimitation may render circumscription of a genus difficult if hybridity has played a role in its origin. Some authors have based genera on the results of cladistic analyses (e.g., Anderberg, 1991), but Stuessy (1990 p. 206) advocated a combination of chronistic (time), cladistic (branching patterns of evolution), patristic (character divergence within lineages) and phenetic (overall similarity) data may provide "the most predictive and useful" discrimination of genera.

1.7 Thesis objectives

The overall aim of the research reported in this thesis was to study intergeneric hybridism among New Zealand Gnaphalieae in order to validate the existence of natural intergeneric hybrids and provide evidence for evolutionary relationships. Using a case-study approach, objectives were to determine the identity of putative hybrids from two study sites, evaluate morphological variation, assess possible reproductive barriers to natural hybridism, and compare the effectiveness of several statistical methods for hybrid identification from morphological data. Experimental crosses were performed with the aim of evaluating relationships among indigenous Gnaphalieae and between indigenous and exotic Gnaphalieae. Other objectives were the collection of data on the breeding systems of the indigenous Gnaphalieae, of which little is known, and evaluation of the fertility of artificial and natural hybrids and the potential for later-generation and backcross hybrids to be produced.

Chapter 2. A Review of Intergeneric Hybridisation in the Compositae

2.1 Introduction

Natural putative intergeneric hybrids in the Compositae have been reported from a wide geographical area, including Britain and continental Europe (see Stace, 1975), Japan (e.g., Inoue, 1961; Tara, 1979), Hawaii (Carr, 1990; Carr, 1995), North America (e.g., Hartman and Lane, 1991) and New Zealand (e.g., Jordan, 1995; Falvey, 1996; Ward, 1997). Several previous surveys have listed intergeneric combinations in the Compositae. Knobloch (1972) listed 31 intergeneric combinations for the Compositae, but the list contains several hybrids of doubtful authenticity, such as *Crepis* L. × *Taraxacum* L. (Stace, 1975). Knobloch gives no indication of the validity or synonomy of the nothotaxon names listed, whether the hybrids were natural or artificial crosses, or of the degree of substantiation for each cross. Some of the hybrids listed are now classified as intrageneric as a result of changes in generic concepts (see section 2.3.2). Stace (1975) listed 13 intergeneric combinations in the Compositae that have arisen naturally in Europe, but he considered the authenticity of some combinations was uncertain and believed two reported combinations (*Filago gallica* L. × *Gnaphalium uliginosum* L. and *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill.) Thell. × *Taraxacum officinale* Weber agg.) were doubtful. Nesom (1994) listed 11 intergeneric combinations, comprising both natural and experimental hybrids, in the tribe Astereae, along with several crosses now treated as intrageneric following reassessments of species relationships.

Intergeneric hybridisation in the Compositae has been investigated to assess generic affinity (e.g., Heiser, 1963; Mitsuoka and Ehrendorfer, 1972; Powell, 1978; Rabakonandrianina and Carr, 1981; Kyhos *et al.*, 1990), to help establish generic limits (e.g., Olorode and Torres, 1970; Powell, 1972; Powell, 1978), to assess the generic affinities of species of uncertain position (e.g., Heiser, 1963; Arnold and Jackson, 1978; Powell, 1978; Stucky, 1978; Bartoli and Tortosa, 1998), to help define subgeneric taxa (e.g., Powell, 1972; Jackson, 1979; Rabakonandrianina and Carr, 1981; Wilcox, 1982), to evaluate the karyotype and chromosome homology in intergeneric hybrids (e.g., Ono, 1951; Ono and Nagai, 1958; Inoue, 1961; Tara, 1979), and to describe natural hybrids or substantiate their identity (e.g., Hartman and Lane, 1991; Heenan, 1993; Jordan, 1995; Falvey, 1996).

Cross-compatibility, hybrid fertility and chromosome homology in intergeneric hybrids have been found to be useful in numerous taxonomic studies in the Compositae and have been important factors in, for example, the relegation of *Pappothrix* and *Laphamia* to sections within *Perityle* (Powell, 1972), the merging of *Tragoceras* with *Zinnia* (Olorode and Torres, 1970), and the transfer of *Aster sonorae* A.Gray to *Machaeranthera* Nees (Stucky, 1978). However, interpretation of such data may be difficult owing to variation in interfertility between different individuals of the same taxa, or the data may conflict with other phylogenetic evidence (e.g., Mitsuoka and Ehrendorfer, 1972). The fertility estimates of putative intergeneric hybrids in the Compositae vary widely, but surprisingly few appear to be completely sterile. Highly fertile later-generation hybrids have been reported in several instances (e.g., Ono, 1955; Carr, 1995) and later-generation (e.g., F_4) hybrids have been raised (e.g., Ono, 1946; Ono, 1950; Ono, 1951; Tara, 1979; Vassilevska-Ivanova *et al.*, 1996).

Artificial intergeneric hybrids have been synthesised in a number of tribes (see Table 2.2 pp. 59–60). Surprisingly, few studies substantiating the parentage of natural putative intergeneric hybrids in the Compositae have been published. Hartman and Lane (1991) presented morphological and anatomical evidence to support the existence of at least one hybrid between *Isocoma veneta* (Kunth) E.Greene and *Xanthocephalum humile* Benth. Jordan (1995) and Falvey (1996) presented morphological, anatomical and biochemical evidence to support the existence of four intergeneric combinations among the New Zealand Gnaphalieae. In a number of instances, detailed morphological descriptions have accompanied publication of nothogeneric names for the putative hybrids (Arènes, 1951; Savelescu *et al.*, 1964; Heenan, 1993).

Detailed investigations of intergeneric hybridisation have been undertaken in several groups in the Compositae. Hybridisation among the tarweeds and Hawaiian silversword alliance (Heliantheae: Madiinae) has helped to resolve phylogenetic relationships. The group comprises 23 genera, including genera recognised by Baldwin (1999), with centres of diversity in California and Hawaii (Kyhos *et al.*, 1990). The occurrence of natural and artificial intergeneric hybrids between the morphologically and ecologically diverse Hawaiian endemic genera *Argyroxiphium*, *Dubautia* and *Wilkesia*, and their partial to full fertility (Kyhos *et al.*, 1990; Carr *et al.*, 1996), indicates they form a close-knit group. Cytogenetic investigation of artificial hybrids has allowed evaluation of genomic relationships in the group (Kyhos *et al.*, 1990). In addition, artificial hybrids between *Dubautia* and tarweed species have been synthesised (Kyhos *et al.*, 1990; Carr *et al.*, 1996), providing evidence for an

ancestral relationship between the Hawaiian and Pacific coast Madiineae, supporting anatomical and molecular evidence (see Baldwin, 1996). Kyhos *et al.* (1990) noted that the potential for intergeneric gene exchange is low among this group, except between *Argyroxiphium* and *Dubautia*, for which four natural intergeneric combinations are recorded (Carr, 1990).

Intergeneric hybridisation in the *Anthemis* group (tribe Anthemideae) has also been investigated experimentally. Natural intergeneric hybrids between *Anthemis*, *Matricaria* and *Tripleurospermum* Sch.Bip. are recorded in Europe (Rothmaler, 1963; Kay, 1971a; Kay, 1971b; Mitsuoka and Ehrendorfer, 1972; Stace, 1975). Mitsuoka and Ehrendorfer (1972) undertook a cytogenetic investigation of artificial intergeneric hybrids to evaluate intrageneric and intergeneric relationships and evolutionary mechanisms in the group. Their results supported the belief that the northern-hemisphere genera *Anthemis*, *Chamaemelum*, *Matricaria* and *Tripleurospermum* form a closely related group, but the results were not always consistent with other evidence. Reciprocal F_1 hybrids between *Anthemis cotula* and *Chamaemelum nobile*, for example, possessed a high level of chromsome pairing and pollen fertility, and backcross hybrids with the maternal parent were produced, suggesting the species have a close phylogenetic affinity; embryological, morphological and phytochemical evidence, however, suggests *Chamaemelum* is more closely related to *Matricaria* (Mitsuoka and Ehrendorfer, 1972; Heywood and Humphries, 1977). The low interfertility of hybrids between *Anthemis* subg. *Anthemis* and subg. *Cota* supported fruit anatomy and phytochemical evidence that indicates *Anthemis* is heterogeneous, and indeed some botanists (e.g., Dostál, 1982) recognise *Cota* at generic level. Species of *Matricaria* were successfully crossed with members of *Anthemis* and *Tripleurospermum* but with great difficulty, suggesting *Matricaria* is more distantly related to *Anthemis* and *Tripleurospermum.* These results support biochemical, embryological and morphological evidence that *Tripleurospermum* is most closely related to *Anthemis*, and *Matricaria* is linked to *Anthemis* through *Chamaemelum* (Kay, 1971a; Mitsuoka and Ehrendorfer, 1972; Heywood and Humphries, 1977). Their results also indicated the annual discoid *Matricaria* species (now placed in the southern-hemisphere genus *Pentzia* Thunb.) have little genetic affinity with northern-hemisphere *Matricaria* species, but their affinity with other *Pentzia* species was not tested. Results also supported the belief that *Matricaria nigellaefolia* DC. and northern-hemisphere *Matricaria* species were only distantly related.

Natural and artificial hybrids involving several Japanaese Lactuceae (*Crepidiastrum keiskeanum* (Maxim.) Nakai, *C. platyphyllum* (Franch. & Savat.) Kitam., *Lactuca indica* L. and *Paraixeris denticulata* (Houtt.) Nakai) were studied by Ono and his co-workers (Ono and Sato, 1935; Ono, 1941; Ono, 1943; Ono, 1946; Ono, 1950; Ono, 1951; Ono and Sakai, 1952; Ono and Sakai, 1954; Ono, 1955; Ono and Nagai, 1958). The cytology, morphology and ovule fertility of the hybrids were investigated. The loss of chromosomes in somatic cells was reported; for example, hybrids between *L. indica* ($2n$ = 18) and *P. denticulata* ($2n$ = 10) would be expected to have $2n$ = 14 chromosomes, but in all nine individuals studied the somatic chromosome number varied from 10 to 14 (Ono, 1943; Ono and Sakai, 1952; Ono and Nagai, 1958). Paternal chromosomes appeared to be eliminated (Ono, 1955). Meiotic abnormalities were also reported in *L. indica* × *P. denticulata* hybrids (Ono, 1943). However, because substantive photographs are largely absent and serial sections were employed, it is difficult to evaluate these studies critically.

Cytological studies of several other intergeneric hybrids in the Compositae have been published. Investigation of the karyotype and meiotic configurations in hybrids between species of *Heteropappus* Less. and *Kalimeris* Cass. provided evidence for a close generic relationship (Inoue, 1961). Inoue concluded that a natural hybrid between *H. arenarius* Kitam. ($2n$ = 36) and *K. yomena* (Kitam.) Kitam. ($2n$ = 63) must be from a later generation than the F_1, as its chromosome number was the same as that of *H. arenarius*, not intermediate as might be expected in F_1 hybrids. Four classes of hybrids between *Aster ageratoides* Turcz. and *Kalimeris incisa* (Fisch.) DC. have been identified based on their karyotypes, reflecting the probable contribution of unreduced gametes (Tara, 1979).

Schilling and Panero (1996) interpreted cpDNA data as suggesting wide hybridisation may have occurred in the past between divergent members of the subtribe Helianthinae. The genera *Helianthus*, *Tithonia* Desf., *Verbesina* L. and *Viguiera* Kunth were considered to be closely related by Heiser *et al.* (1969) and the successful production of artificial hybrids between members of these genera (Heiser *et al.*, 1969; Christov and Panayotov, 1991; Vassilevska-Ivanova *et al.*, 1996) demonstrates wide crosses between distinct, extant lineages in this subtribe are still possible.

Natural intergeneric hybrids are recorded among the New Zealand Astereae and Gnaphalieae. Four combinations are recorded between species of *Celmisia* and *Olearia* (Clarkson, 1988; Heenan, 1993) but are very rare. The pollen stainability of *C. gracilenta* Hook.f. × *O.*

arborescens (G.Forst.) Cockayne & Laing hybrids is low (Heenan, 1993). Natural hybrids are also recorded between *Damnamenia* and *Pleurophyllum* (Given, 1973).

The occurrence of natural intergeneric hybrids among the New Zealand Gnaphalieae was first proposed early last century (Cockayne, 1922; Cockayne and Allan, 1926). Natural putative intergeneric hybrids between indigenous *Anaphalioides*, *Euchiton*, *Ewartia*, *Helichrysum*, *Leucogenes* and *Raoulia* species have been collected, but none involving *Craspedia*, *Ozothamnus* and *Pseudognaphalium* are recorded (Ward, 1997). However, little evidence has been published to substantiate their existence. Allan (1939) investigated the leaf venation and tomentum in several putative *Leucogenes* × *Raoulia* hybrids. Drury (1972) presented morphological evidence in support of his determination of plants from the Tararua Range, Wellington as hybrids between *Euchiton mackayi* and *Leucogenes leontopodium.* Recently, Jordan (1995) and Falvey (1996) presented morphological, anatomical and leaf flavonoid evidence for the occurrence of the following natural hybrids: *Anaphalioides bellidioides* × *Helichrysum lanceolatum*, *A. bellidioides* × *Leucogenes grandiceps*, *A. bellidioides* × *Raoulia mammillaris*, and *L. grandiceps* × *R. mammillaris.* Putative hybrids between *A. bellidioides* and *Ewartia sinclairii* have also been collected (Allan, 1961) and are the focus of investigation later in this thesis (see Chapter 4). A hybrid between *A. bellidioides* and *H. intermedium* Simpson var. *tumidum* Cheeseman was raised from field-collected seed and has been given the cultivar name 'Graeme Paterson' (Heenan, 1989). Another putative hybrid between *A. bellidioides* and *H. intermedium* in cultivation has been given the cultivar name 'Ashley Forest'. Putative hybrids representing numerous other intergeneric combinations have been reported for the New Zealand Gnaphalieae but not investigated in detail. Glenny (1997) listed *A. bellidioides* × *L. leontopodium*, *A. bellidioides* × *R. glabra* Hook.f., *A. subrigida* (Colenso) Anderb. × *R. tenuicaulis* Hook.f., and possible hybrids between *A. bellidioides* and *R. hookeri* Allan, and *A. bellidioides* and *H. intermedium.* Other putative combinations include *H. dimorphum* Cockayne × *R. glabra* (Brockie, 1956), *H. depressum* (Hook.f.) Benth. & Hook.f. × *R. tenuicaulis* (Allan, 1961), *H. filicaule* Hook.f. × *R. glabra* (Druce, 1971) and *H. intermedium* × *L. grandiceps* (Molloy, 1980). Allan (1961) regarded hybrids between *A. bellidioides* and *H. filicaule* as "suspected rather than established" and, following a recent revision of *Anaphalioides*, Glenny (1997) did not examine any specimens suggesting such a hybrid origin. Cockayne and Allan (1934) listed the cross *H. filicaule* × *H. lanceolatum* without comment. Allan (1961) referred to putative hybrids between *Pseudognaphalium luteoalbum* and the adventive *Gamochaeta purpurea* (L.) Cabrera (syn. *Gnaphalium*

purpureum L.), but these specimens have subsequently been determined as *Gamochaeta purpurea* (Drury, 1971; Webb, 1988). No putative intergeneric hybrids involving indigenous *Craspedia*, *Ozothamnus* and *Pseudognaphalium* have been collected (Ward, 1997).

Because of their distinct morphology, several putative intergeneric hybrids among New Zealand Gnaphalieae were described as species (Table 2.1 p. 36). The first intergeneric hybrids to be collected, *Leucogenes leontopodium* × *Raoulia rubra* from the Tararua Range, Wellington, were described as *Haastia loganii* by Buchanan (1882). After being transferred to *Helichrysum* by Kirk (1899) then to *Raoulia* by Cheeseman (1925), their hybrid origin was recognised by Allan (1939). Other putative hybrids between *Leucogenes* species and pulvinate species of *Raoulia* subg. *Psychrophyton* to have been described as species are *L. grandiceps* × *R. bryoides* (*Raoulia gibbsii*), *L. grandiceps* × *R. mammillaris* (*Helichrysum pauciflorum*) and *L. leontopodium* × *R. grandiflora* (*Gnaphalium* (*Helichrysum*) *fasciculatum*) (Molloy, 1980). Hybrids between *Anaphalioides bellidioides* and *Helichrysum lanceolatum* were described as *Helichrysum purdiei* Petrie (Petrie, 1890). Its validity as a species was not questioned until a hybrid swarm was discovered near Hanmer Springs (Cockayne, 1922; Cockayne and Allan, 1926). It was subsequently listed as a hybrid (e.g., Cockayne and Allan, 1934), but no supporting evidence was presented until the recent study of Jordan (1995). Webb (1988 p. 251) described *A. bellidioides* × *H. lanceolatum* hybrids as "widespread and common". Putative *A. bellidioides* × *Ewartia sinclairii* hybrids (see Chapter 4) and *Helichrysum intermedium* × *L. grandiceps* hybrids were also described as species.

2.2 Hybrid nomenclature

The application of hybrid formulae and names is governed by the *International Code of Botanical Nomenclature* (Greuter *et al.*, 2000) and the *International Code of Nomenclature of Cultivated Plants* (Trehane *et al.*, 1995). If its parentage is known, a hybrid can be designated by a 'hybrid formula' comprising the names of the parental taxa linked by a multiplication symbol. If the direction of the cross is known, the female parent precedes the male parent; for example, the cultivated intergeneric hybrid 'Graeme Paterson' has the hybrid formula *Helichrysum intermedium* var. *tumidum* × *Anaphalioides bellidioides*. When the sexes are unknown, the parental taxa are listed alphabetically, e.g., *Anaphalioides bellidioides* × *Ewartia sinclairii*. The naming of hybrids is not obligatory but is recommended when the use of such names is more convenient or less cumbersome than hybrid formulae. Numerous natural intergeneric hybrids in the Compositae have been given taxonomic names.

Putative hybrid	Species names	References
Anaphalioides bellidioides × *Ewartia sinclairii*	*Helichrysum fowerakeri* Cockayne, Trans. N. Z. Inst. 48: 196 (1916)	Cockayne and Allan (1934); Allan (1961)
Anaphalioides bellidioides × *Helichrysum lanceolatum*	*Helichrysum purdiei* Petrie, Trans. N. Z. Inst. 22: 440 (1890)	Cockayne and Allan (1934); Allan (1961); Jordan (1995)
Helichrysum intermedium × *Leucogenes grandiceps*	*Helichrysum* (*Leucogenes*) *grahamii* Petrie, Trans. N. Z. Inst. 45: 268 (1913) *Leucogenes grahamii* (Petrie) Cheeseman, Manual N. Z. Fl.: 980 (1925)	Allan (1939, 1961); Molloy (1980)
Leucogenes grandiceps × *Raoulia bryoides*	*Raoulia gibbsii* Cheeseman, Trans. N. Z. Inst. 42: 216 (1910)	Allan (1939, 1961); Molloy (1980)
Leucogenes grandiceps × *Raoulia mammillaris*	*Helichrysum pauciflorum* Kirk, Trans. N. Z. Inst. 27: 351 (1895)	Allan (1939, 1961); Molloy (1980); Falvey (1996)
Leucogenes leontopodium × *Raoulia grandiflora*	*Gnaphalium* (*Helichrysum*) *fasciculatum* Buchanan, Trans. N. Z. Inst. 9: 529 (1876) *Helichrysum fasciculatum* (Buchanan) Kirk, Student's Fl. N. Z.: 310 (1899) *Raoulia grandiflora* var. *fasciculatum* (Buchanan) Cheeseman, Manual N. Z. Fl.: 974 (1925)	Allan (1939, 1961); Molloy (1980)
Leucogenes leontopodium × *Raoulia rubra*	*Haastia loganii* Buchanan, Trans. N. Z. Inst. 14: 350 (1882) *Helichrysum loganii* (Buchanan) Kirk, Student's Fl. N. Z.: 310 (1899) *Raoulia loganii* (Buchanan) Cheeseman, Manual N. Z. Fl.: 972 (1925)	Allan (1939, 1961)

Table 2.1. Putative intergeneric hybrids in the New Zealand Gnaphalieae that have been described as species.

A list of all such names of which I am aware and comments on their validity is presented in section 2.3.

Hybrids with names can be designated by prefixing the term 'notho' to the rank of the taxon. Thus hybrids between two particular species can be designated 'nothospecies' and a 'nothogenus' encompasses all hybrids between two specific genera. The general term 'nothotaxon' may also be used for all categories. Hybrids can be given a binary name similar to that of species. For intrageneric hybrids, the binomial consists of the genus name and a 'collective epithet' preceded by a multiplication symbol, e.g., *Helichrysum* ×*selago* (Hook.f.) Benth. & Hook.f. designates all hybrids derived from crosses between *H. coralloides* (Hook.f.) Benth. & Hook.f. and *H. parvifolium* Yeo. The collective epithet is equivalent to a species epithet and is governed by the same rules regarding its formation, publication and use. For an epithet published on or after 1 January 1935 to be validly published, it must be accompanied by a Latin description or diagnosis, or a reference to a previously validly published description or diagnosis, and a type specimen must be designated. A name published without a Latin description or diagnosis is termed a 'nomen nudum'. Where more than one name has been validly published for a taxon, the earliest published name is the correct name; the later names are nomenclaturally superfluous and treated as synonyms. If the same name has been published but is based on a different type specimen, it is termed a homonym. Superfluous names that are not validly published are termed 'illegitimate'. Epithets originally published as species or intrageneric taxa, but which are later found to be nothotaxa, can be retained and hybridity indicated by placing a multiplication symbol before the epithet; the author citation remains unchanged but the original category may be indicated in parentheses, e.g., *Helichrysum* ×*selago* (Hook.f.) Benth. & Hook.f. (pro sp.). Collective epithets combining with a hyphen the unaltered epithets of the parental taxa, or with the termination of only one epithet changed, or combining the specific epithet of one parent with the generic name of the other parent, are deemed to be formulae and not true epithets and are thus not validly published.

For intergeneric hybrids the first name of the binomial is known as a 'condensed formula' or 'nothogenus' and is equivalent to a genus. It combines part or the whole of each parental generic name into a single word and is preceded by a multiplication symbol, e.g., the condensed formula ×*Leucoraoulia* encompasses all hybrids between *Leucogenes* and *Raoulia* species. Some 43 other condensed formulae have been published for intergeneric hybrids in the Compositae alone (see section 2.3). For hybrids involving four or more genera, the

condensed formula is derived from a person's name with the ending *-ara*, e.g., ×*Brilliandeara* (= *Aspasia* Lindl. × *Brassia* R.Br. × *Cochlioda* Lindl. × *Miltonia* Lindl. × *Odontoglossum* HBK. × *Oncidium* Sw.). Valid publication of a nothogenus only requires the condensed formula to be accompanied by a statement of the names of the parental genera, so no description, diagnosis or type specimen is required. If the generic name of one or both parental taxa changes, publication of a new nothogeneric name is required to reflect this. As for intrageneric hybrids, the second name of the binomial is a collective epithet.

Hybrids can also be given a cultivar or clonal name without a collective epithet, as in the case of a cultivated hybrid between *Helichrysum intermedium* var. *tumidum* and *Anaphalioides bellidioides*, which has been given the cultivar name 'Graeme Paterson' (Heenan, 1989). Another hybrid between *A. bellidioides* and *H. intermedium* is cultivated under the name 'Ashley Forest'.

A thorough literature search was undertaken with the aim of assessing the extent of intergeneric hybridisation in the Compositae. A list of putative intergeneric hybrids that reflect the generic concepts of Tutin *et al.* (1976) and Bremer (1994) was compiled, along with a comprehensive list of published nothotaxon names with determinations of their validity.

2.3 Synopsis of artificial and natural putative intergeneric hybrids in the Compositae reported in botanical literature

The following lists of experimental and putative natural intergeneric hybrids were compiled from *Index Kewensis*, other published sources and theses. Decisions on their authenticity are based only on published evidence and inferences of other authors. Some of the hybrids are well documented and have been investigated experimentally; some are probably authentic but little or no substantive evidence has been published; others are doubtful or erroneous. In several instances intrageneric hybrids have initially been misidentified as being of intergeneric origin. For all hybrid formulae, the parental genera are given in alphabetic order, rather than the maternal parent preceding the paternal parent. Because many of the nothotaxon names are not legitimately published or no longer correct due to changes in generic concepts, published names are listed in chronological order under each hybrid formula and comments on their validity are made under 'Notes'. Some of the hybrids listed may be classified as either intrageneric or intergeneric depending on the generic concepts followed. The generic concepts of Tutin *et al.* (1976) and Bremer (1994) were usually followed when compiling the lists, but

some deviations from these by other authors are noted. Author abbreviations follow Brummitt and Powell (1992); wherever possible, book abbreviations follow Stafleu and Cowan (1976-1988) and Stafleu and Mennega (1992-2000); and journal abbreviations follow Lawrence *et al.* (1968) and Alkire (1998). In several instances citations are incomplete, as I have been unable to ascertain the place of publication. Where the earliest valid publication of a name is unknown, the citation used by *Index Kewensis* or other authors is followed and the need for a further literature search is noted. The tribe to which each hybrid belongs is listed beside the condensed formula and follows Bremer (1994).

The following symbols or abbreviations are used in the lists below: syn., the name is a nomenclatural synonym; †, reference seen; ‡, reference not seen; ⊕, the name appears to be validly published (although no type specimens have been examined); ⊗, the name was not validly published in this reference. Note that when placed after a binomial, the latter two symbols refer to the collective epithet.

2.3.1 Intergeneric hybrids with nothotaxon names

***Achillea* L. × *Tanacetum* L.** ANTHEMIDEAE

Natural hybrids:

A. setacea Waldst. & Kit. × *Tanacetum millefolium* (L.) Tzvelev (syn. *Chrysanthemum millefolium* L.)

Chrysanthemoachillea ×*borzae* Prodán, Achil.: 50 (1931) ‡ ; Prodán ex Nyár., in Savul., Fl. Reipubl. Popul. Roman. 9: 408, 964 (1964) †⊕

A. pannonica Scheele × *T. millefolium* (L.) Tzvelev

Chrysanthemoachillea ×*carmen-sylvae* Prodán ex Nyár., in Savul., Fl. Reipubl. Popul. Roman. 9: 408, 964 (1964) †⊕

Notes:

Tanacetum millefolium is no longer placed in *Chrysanthemum* (e.g., Tutin *et al.*, 1976), hence to reflect this transfer, publication of a new condensed formula combining the names *Achillea* and *Tanacetum* is required.

The author citations given follow *Index Kewensis*. The condensed formula ×*Chrysanthemoachillea* was not validly published in Savelescu *et al.* (1964) as it was not preceded by a multiplication symbol, and I have not seen the earlier publication by Prodán. However, it is validly published elsewhere (e.g., Stace, 1975). A literature search is thus

required to determine the earliest valid publication of this name. The collective epithets are validly published but publication of new combinations is required.

***Anthemis* L. × *Matricaria* L.** ANTHEMIDEAE

Anthemi-Matricaria P.Fourn., Fl. Compl. Plaine Franç.: 316 (1928) †⊗

×*Anthematricaria* Geisenh. ex Domin, Preslia 13-15: 231 (1935) †⊗

Natural hybrid:

A. cotula L. × *M. recutita* L. (syn. *Chamomilla recutita* (L.) S.F.Gray)

Anthe-Matricaria dominii Rohlena, Cas. Národ. Mus. 104: 9 (1930) †⊕

×*Anthechamomilla dominii* (Rohlena) Rauschert, Feddes Repert. 93: 10 (1983) †

Experimental hybrid:

Mitsuoka and Ehrendorfer (1972).

Additional reference: Stace (1975).

Notes:

In Fournier (1928) the condensed formula *Anthemi-Matricaria* was not preceded by a multiplication symbol when first used (on p. 273), although it was on p. 274. This could be interpreted as an orthographic error, but in a footnote on p. 316 Fournier acknowledged his nothogeneric names did not comply with the botanical code. It is thus clear the initial form was Fournier's intention and hence under the current botanical code '×*Anthemimatricaria*' was not validly published by Fournier (1928).

The author citation for ×*Anthematricaria* follows *Index Kewensis*, but the condensed formula was not preceded by a multiplication symbol in Domin (1935). The name is validly published elsewhere (e.g., Stace, 1975), thus a literature search is required to determine the earliest valid publication of this name.

Kay (in Tutin *et al.*, 1976) recognised *Chamomilla* S.F.Gray, in which case ×*Anthechamomilla dominii* (Rohlena) Rauschert would be the correct name for this hybrid.

***Anthemis* L. × *Tripleurospermum* Sch.Bip.** ANTHEMIDEAE

×*Anthepleurospermum* Rothm., Exkursionsflora von Deutschland IV: 324 (1963) †⊕

×*Tripleurothemis* Stace, Watsonia 18: 212 (1990) †⊕

Natural hybrids:

A. cotula L. × *T. inodorum* Sch.Bip.

Anthe-Matricaria celakovskyi Geisenh. (1890) ‡

Anthemi-Matricaria maleolens P.Fourn., Fl. Compl. Plaine Franç. 274 (1928) †⊕

Anthechrysanthemum celakovskyi Geisenh. ex Domin, Preslia 13-15: 233 (1935) †

×*Anthepleurospermum celakovskyi* (Geisenh. ex Domin) Rothm., loc. cit. †

×*Tripleurothemis maleolens* (P. Fourn.) Stace, loc. cit. †

A. tinctoria L. × *T. inodorum* Sch.Bip.

Anthe-Matricaria hampeana Geisenh. loc. cit. ‡

Anthemi-Matricaria sulfurea P.Fourn., loc. cit. †⊕

×*Anthepleurospermum hampeanum* (Geisenh. ex Domin) Rothm., loc. cit. †

A. arvensis L. × *T. inodorum* Sch.Bip.

Anthe-Matricaria gruetteriana Asch.(1890) ‡

Anthemi-Matricaria inolens P.Fourn., loc. cit. †⊕

×*Anthepleurospermum gruetterianum* (Geisenh. ex Domin) Rothm., loc. cit. 325 †

Experimental hybrids:

A. altissima L. × *T. tetragonospermum* (F.Schmidt) Pobed.

A. cotula L. × *T. tetragonospermum* (F.Schmidt) Pobed.

Additional references: Kay (1971a, 1971b); Mitsuoka and Ehrendorfer (1972); Stace (1997).

Notes:

×*Anthepleurospermum* Rothm. is the earliest validly published condensed formula for hybrids between *Anthemis* and *Tripleurospermum*, hence ×*Tripleurothemis* Stace must be considered a synonym.

Kay (in Stace, 1975) considered the collective epithets published by Geisenheyner and Ascherson in 1890 to be invalidly published, but I have not seen the original references. Collective epithets were validly published by Fournier (1928) and Stace (1990) accepted the epithet '*maleolens*' as the earliest valid name for hybrids between *A. cotula* and *T. inodorum*. Rothmaler, however, used the collective epithets of Geisenheyner and Ascherson when publishing the name ×*Anthepleurospermum*. Thus if the epithets of Ascherson and Geisenheyner are invalidly published, the transfer of Fournier's collective epithets to ×*Anthepleurospermum* is required.

***Argyroxiphium* DC. × *Dubautia* Gaudich.** HELIANTHEAE

×*Argyrautia* Sherff, Amer. J. Bot. 31: 159 (1944) †⊕

Natural hybrids:

A. grayanum (Hillebr.) O.Deg. × *D. laxa* Hook. & Arn.

×*Argyrautia degeneri* Sherff, loc. cit. 160 †⊕

A. grayanum (Hillebr.) O.Deg. × *D. dolosa* (O.Deg. & Sherff) G.D.Carr

A. grayanum (Hillebr.) O.Deg. × *D. scabra* (DC.) D.D.Keck subsp. *leiophylla* (A.Gray) G.D.Carr

A. kauense (Rock & M.Neal) O.Deg. & I.Deg. × *D. ciliolata* (DC.) D.D.Keck

A. sandwicense DC. subsp. *macrocephalum* (A.Gray) A.Meyrat × *D. menziesii* (A.Gray) D.D.Keck

Experimental hybrids:

Carr and Kyhos (1986); Baldwin (1996).

Additional references: Carr (1990, 1995).

***Aster* L. × *Erigeron* L.** ASTEREAE

×*Asterigeron* Tzvelev, Fl. Evropeiskoi Chasti SSSR 7: 185 (1994) †⊕

Natural hybrid:

A. amellus L. × *E. acris* L.

×*A. ucrainicus* Tzvelev, loc. cit. †

Notes:

The name '*Asterigeron*' was published as a monotypic genus by Rydberg (1918) to accommodate the species *Aster watsonii* A.Gray. Cronquist (1947) transferred this species to *Erigeron*, but Grau (1977) lists *Asterigeron* Rydb. as a synonym of *Aster*. Under the current botanical code ×*Asterigeron* Tzvelev is a homonym of *Asterigeron* Rydb., hence publication of a new condensed formula for the above hybrid is required.

***Carduus* L. × *Cirsium* L.** CARDUEAE

×*Carduocirsium* Sennen ex P.Fourn., Quartre Fl. France: 1004 (1940) †⊕

×*Cirsiocarduus* P.Fourn. ex Arènes, Mém. Mus. Hist. Nat. Paris, n. s. 24: 253 (1949)†⊕

Natural hybrids:

Carduus carlinifolius Lam. × *Cirsium monspessulanum* (L.) All.

×*Carduocirsium guetrotii* Sennen, in Guétrot, Pl. Hybr. Fr. I & II: 30 (1925) †⊕

Carduus crispus L. × *Cirsium monspessulanum* (L.) All.

×*Carduocirsium fanii* Sennen, in Guétrot, loc. cit. 29 (1925) †⊕

Carduus defloratus subsp. *carduelis* (L.) Gugler × *Cirsium eristhales* (Jacq.) Scop.

Carduus cirsiformis Vuk., Consp. Fl. Eur., Suppl. II, 1: 183 (1889) ‡

×*Cirsiocarduus cirsiformis* (Vuk.) Arènes, loc. cit. †

Carduus defloratus subsp. *carlinifolius* (Lam.) Bonnier × *Cirsium acaule* Scop.

× *Cirsium jaubertianum* Sennen & Septimin, Bull. Soc. Bot. France 73: 655 (1926), in obs. ‡

×*Cirsiocarduus jaubertianus* (Sennen & Septimin) Arènes, loc cit. 254 †

Carduus nigrescens subsp. *uncinatus* × *Cirsium eriophorum* subsp. *turkestanicum*

×*Cirsiocarduus hohenackeri* Arènes, loc. cit. 255 †

Carduus nutans L. × *Cirsium monspessulanum* (L.) All.

Carduus borderi Rouy, Fl. Fr. 9: 92 (1905) ‡

×*Carduocirsium borderei* (Rouy) P.Fourn., loc. cit. †

×*Cirsiocarduus borderi* (Rouy) Arènes, loc. cit. †

Carduus nutans L. × *Cirsium vulgare* subsp. *savianum* Arènes

Carduus parisiensis E.G. Camus ‡

Cirsio-Carduus parisiensis P.Fourn, Fl. Compl. Plaine Franç.: 277 (1928) †

×*Carduocirsium parisiense* (E.G. Camus) P.Fourn., Quartre Fl. Fr.: 1004 (1940) †

Carduus personatus (L.) Jacq. × *Cirsium heterophyllum* (L.) All.

Carduus khekii Petitm., Monde Plant. 39: 22 (1906) ‡

×*Carduocirsium khekii* (Petitm.) P.Fourn., Quartre Fl. Fr.: 1004 (1940) †

Carduus pycnocephalus L. × *Cirsium vulgare* (Savi) Ten.

Carduus tenuiflorus Curt. × *Cirsium vulgare* subsp. *savianum* Arènes

×*Cirsiocarduus lutetianus* Arènes, Not. Syst. 14: 194 (1952) †⊕

Additional references: Sennen (1931); Sennen and Fournier (1933); Allan (1940a); Stace (1975).

Notes:

×*Carduocirsium* Sennen ex P.Fourn. is the earliest validly published condensed formula for hybrids between *Carduus* and *Cirsium*. The name was not validly published by Sennen (in Guétrot, 1927) or Sennen (1931) as it was not preceded by a multiplication symbol. The condensed formula ×*Cirsiocarduus* was not validly published by Fournier (1928) as it was not preceded by a multiplication symbol, but it was validly published by Arènes (1949).

New combinations in ×*Carduocirsium* are required for the following four nothospecies that currently only have names available in ×*Cirsiocarduus*: ×*C. cirsiformis*, ×*C. hohenackeri*, ×*C. jaubertianus* and ×*C. lutetianus*.

The cross *Carduus pycnocephalus* × *Cirsium vulgare* was reported from New Zealand by Allan (1940a), where the two species are adventive.

Despite the number of putative hybrids that have been named, Sledge (in Stace, 1975) considered hybrids between *Carduus* and *Cirsium* from continental Europe were of doubtful authenticity.

***Carduus* L. × *Galactites* Moench** CARDUEAE

×*Carduogalactites* P.Fourn., Quartre Fl. France: 1001 (1940) †⊕

Natural hybrid:

C. pycnocephalus L. × *G. tomentosa* Moench

Galactites ×*ludoviciae* Bertr., Bull. Acad. Int. Géogr. Bot. 21: 294 (1911) ‡

×*Carduogalactites ludoviciae* (Bertr.) P.Fourn., loc. cit. †

***Celmisia* Cass. × *Olearia* Moench** ASTEREAE

×*Celmearia* Heenan, Horticulture New Zealand 4: 2 (1993) †⊕

Natural hybrids:

C. durietzii Cockayne & Allan ex W.Martin × *O. avicenniifolia* (Raoul) Hook.f.

C. gracilenta Hook.f. × *O. arborescens* (G.Forst.) Cockayne & Laing

×*C. ruawahia* Heenan, loc. cit. †⊕

C. incana Hook.f. × *O. arborescens* (G.Forst.) Cockayne & Laing

C. spectabilis Hook.f. × *O. arborescens* (G.Forst.) Cockayne & Laing

Additional reference: Clarkson (1988).

***Centaurea* L. × *Serratula* L.** CARDUEAE

×*Centauserratula* Arènes, Not. Syst. 14: 188 (1952) †⊕

Natural hybrid:

C. pygmaea Benth. × *S. cerinthefolia* Sibth. & Sm.

×*C. mouterdei* Arènes, loc. cit. †⊕

Additional reference: Stace (1975).

***Conyza* Less. × *Erigeron* L.** ASTEREAE

×*Conyzigeron* Rauschert, Feddes Repert. 83: 656 (1973) †⊕

×*Conygeron* Holub, Fol. Geobot. Phytotax. 8: 156, 176 (1973) †⊕

Natural hybrid:

C. canadensis (L.) Cronquist × *E. acris* L.

Erigeron huelsenii Vatke, Österr. Bot. Z. 21: 346 (1881) ‡

E. ×*riualbensis* Nyár. ‡

×*Conyzigeron huelsenii* (Vatke) Rauschert, loc. cit. †

×*Conygeron huelsenii* (Vatke) Holub, loc. cit. †

Additional references: Stace (1975); Dostál (1982); Stace (1997).

Note:

Publication of the condensed formula ×*Conyzigeron* Rauschert preceded that of ×*Conygeron* Holub and hence is the correct name for hybrids between *Conyza* and *Erigeron*.

***Filago* Cass. × *Logfia* Cass.** GNAPHALIEAE

Natural hybrid:

Filago vulgaris Lam. (syn. *Gifola vulgaris* Cass.) × *Logfia arvensis* (L.) Holub (syn. *Oglifa arvensis* Cass.)

Filago mixta Holuby, Österr. Bot. Z. 21: 261 (1871) ‡

×*Giflifa mixta* (Holuby) Chrtek & Holub, Preslia 35: 12 (1963) †

Additional reference: Tutin *et al.* (1976).

Note:

Chrtek and Holub (1963) published the condensed formula ×*Giflifa* Chrtek & Holub for this hybrid, but the putative parental species are placed in *Filago* and *Logfia* by Holub (in Tutin *et al.*, 1976) and Anderberg (1994). Thus to reflect their transfer, publication of a new condensed formula combining the names *Filago* and *Logfia* is required.

***Heteropappus* Less. × *Kalimeris* Cass.** ASTEREAE

×*Heterokalimeris* Kitam., Acta Phytotax. Geob. 8: 195 (1939) †⊗

Natural hybrids:

H. arenarius Kitam. × *K. yomena* (Kitam.) Kitam.

×*Heterokalimeris maruyamae* Kitam., loc. cit. †⊕

H. hispidus (Thunb.) Less. × *K. incisa* (Fisch.) DC.

Experimental hybrids:

H. arenarius Kitam. × *K. incisa* (Fisch.) DC.

H. hispidus (Thunb.) Less. × *K. indica* (L.) Sch.Bip.

Additional references: Huziwara (1950); Inoue (1961).

Notes:

Bremer (1994) accepted both *Heteropappus* and *Kalimeris*, but Iwatsuki *et al.* (1993) included both genera in a broad concept of *Aster*.

The condensed formula ×*Heterokalimeris* was not validly published by Kitamura (1939b), as it was not preceded by a multiplication symbol, but it has been legitimately published elsewhere (e.g., Iwatsuki *et al.*, 1993). Thus a literature search is required to determine the earliest valid publication of this name.

Ixeris Cass. × _Youngia_ Cass. LACTUCEAE

×*Ixyoungia* Kitam., Acta Phytotax. Geob. 11: 131 (1942) †⊗

Natural hybrids:

I. debilis (Thunb. ex Murray) A.Gray × *Y. japonica* (L.) DC.

×*I. sekimotoi* Kitam., loc. cit. †⊕

I. stolonifera A.Gray × *Y. japonica* (L.) DC.

×*I. yendoi* Kitam., loc. cit. †⊕

Note:

The nothogenus ×*Ixyoungia* was not validly published by Kitamura (1942), as it was not preceded by a multiplication symbol, but it has been legitimately published elsewhere (e.g., Iwatsuki *et al.*, 1993). Thus a literature search is required to determine the earliest valid publication of this name.

Lapsana L. × _Youngia_ Cass. LACTUCEAE

×*Lapsyoungia* Hiyama, J. Jap. Bot. 28: 218 (1953) †⊕

Natural hybrid:

L. humilis (Thunb.) Makino × *Y. japonica* (L.) DC.

Lapsana musashiensis Hiyama, J. Jap. Bot. 26: 224 (1951) ‡

×*Lapsyoungia musashiensis* (Hiyama) Hiyama, loc. cit. (1953) †

Additional references: Ohwi (1965); Iwatsuki *et al.* (1993).

***Leucanthemum* Mill. × *Tanacetum* L.** ANTHEMIDEAE

×*Leucantanacetum* Rauschert, Feddes Repert. 83: 656 (1973) †⊕

Natural hybrid:

L. vulgare L. × *T. corymbosum* (L.) Sch.Bip.

Chrysanthemum rohlenae Domin, Repert. Nov. Spec. Regni Veg. 1: 14 (1905) †⊕

×*Leucopyrethrum rohlenae* (Domin) Dostál, Seznam cévnatych rostlin kveteny ceskoslovenské: 267 (1982) †

×*Leucantanacetum rohlenae* (Domin) Rauschert, loc. cit. †

***Leucogenes* Beauverd × *Raoulia* Hook.f.** GNAPHALIEAE

×*Leucoraoulia* Cockayne & Allan, in Cockayne, Veg. New Zealand: 284 (1928) †⊕

Natural hybrids:

L. grandiceps (Hook.f.) Beauverd × *Raoulia bryoides* Hook.f.

Raoulia gibbsii Cheeseman, Trans. N. Z. Inst. 42: 216 (1910) †⊕

L. grandiceps (Hook.f.) Beauverd × *Raoulia eximia* Hook.f.

L. grandiceps (Hook.f.) Beauverd × *Raoulia goyenii* Kirk

L. grandiceps (Hook.f.) Beauverd × *Raoulia mammillaris* Hook.f.

Helichrysum pauciflorum Kirk, Trans. N. Z. Inst. 127: 351 (1895) †⊕

L. leontopodium (Hook.f.) Beauverd × *R. grandiflora* Hook.f.

Gnaphalium (*Helichrysum*) *fasciculatum* Buchanan, Trans. N. Z. Inst. 9: 529 (1876) †⊕

Raoulia grandiflora var. *fasciculatum* (Buchanan) Cheeseman, Manual New Zealand Fl.: 974 (1925) †

L. leontopodium (Hook.f.) Beauverd × *R. rubra* Buchanan

Haastia loganii Buchanan, Trans. N. Z. Inst. 14: 350 (1881) †⊕

Helichrysum loganii (Buchanan) Kirk, Student's Fl. New Zealand: 310 (1899) †

Raoulia loganii (Buchanan) Cheeseman, Manual New Zealand Fl.: 972 (1925) †

Additional references: Allan (1939, 1961); Molloy (1980); Falvey (1996).

Notes:

Despite presenting evidence for the hybrid origin and parentage of the above putative hybrids, Allan (1939, 1961) and Molloy (1980) did not make new combinations in ×*Leucoraoulia* for the epithets listed above.

The putative parentage listed for each published name follows Molloy (1980).

Anderberg (1991) resurrected the genus *Psychrophyton* Beauverd, but the concept of *Raoulia* follows Ward and Breitwieser (1998a), who retained *Raoulia sensu lato* pending resolution of the relationships of the constituent taxa.

***Ligularia* Cass. × *Parasenecio* W.W.Smith & J.Small** SENECIONEAE

Natural hybrid:

L. fischeri (Ledeb.) Turcz. (syn. *Senecillis fischeri* (Ledeb.) Kitam.) × *Parasenecio delphiniifolia*

(Siebold & Zucc.) H.Koyama (syn. *Cacalia delphiniifolia* Siebold & Zucc.)

Ligularia telphusiformis Koidz., Bot. Mag. Tokyo 37: 57 (1923) ‡

×*Senecillicacalia telphusiformis* (Koidz.) Kitam., Acta Phytotax. Geob. 8: 89 (1939) †

Additional references: Ohwi (1965); Iwatsuki *et al.* (1993).

Notes:

The parentage given follows Iwatsuki *et al.* (1993). To reflect the transfer of the parental taxa to *Ligularia* and *Parasenecio*, publication of a new condensed formula combining the two generic names is required.

The nothogenus ×*Senecillicacalia* was not validly published by Kitamura (1939a), as it was not preceded by a multiplication symbol, but it has been legitimately published elsewhere (e.g., Iwatsuki *et al.*, 1993). Thus a literature search is required to determine the earliest valid publication of this name.

***Macroclinidium* Maxim. × *Pertya* Sch.Bip.** MUTISIEAE

Macropertya Honda, Bot. & Zool. 5: 152 (1937) †⊗

Natural hybrid:

M. robustum Maxim. (syn. *P. robusta* (Maxim.) Beauverd) × *P. scandens* (Thunb.) Sch.Bip.

Pertya hybrida Makino, Bot. Mag. Tokyo 14: 144 (1900) ‡

Macropertya hybrida (Makino) Honda, loc. cit. 153 †

Additional reference: Ohwi (1965).

Notes:

Macroclinidium is included by some authors (e.g., Ohwi, 1965; Iwatsuki *et al.*, 1993) in *Pertya*, in which case hybrids would be intrageneric.

The nothogenus *Macropertya* was not validly published by Honda (1937), as it was not preceded by a multiplication symbol. A literature search is required to determine whether the name has been validly published.

***Matricaria* L. × *Tripleurospermum* Sch.Bip.** ANTHEMIDEAE

Pseudomatricaria Domin, Preslia 13-15: 233 (1935) †⊗

Natural hybrid:

M. recutita L. (syn. *Chamomilla recutita* (L.) S.F.Gray) × *T. inodorum* (L.) Sch.Bip.

Pseudomatricaria rohlenae Domin, loc. cit., nom. nud. †⊗

Matricaria ×*rohlenae* (Domin) Dostál, Kvetena CSR: ? (1950) ‡

Experimental hybrid:

M. recutita L. × *T. tetragonospermum* (F.Schmidt) Pobed.

Additional references: Mitsuoka and Ehrendorfer (1972); Stace (1975).

Notes:

The nothogenus *Pseudomatricaria* was not validly published by Domin (1935), as the name was not preceded by a multiplication symbol. A literature search is required to determine whether the name has been validly published. In addition the collective epithet '*rohlenae*' was not validly published by Domin, as a description or Latin diagnosis was lacking.

Under the *Rules of Botanical Nomenclature* current at the time (Briquet, 1935), '*Pseudomatricaria*' was a valid nothogeneric name. In 1966 the requirement for condensed formulas to combine part or all of each parental generic name was introduced (Lanjouw *et al.*, 1966). Since no starting date is specified for this regulation, it is here interpreted as being retroactive. Thus publication of a new condensed formula combining the names *Matricaria* and *Tripleurospermum* is required. *Chamomilla* S.F.Gray is recognised by Kay (in Tutin *et al.*, 1976) but not by Bremer (1994).

***Prenanthes* L. × *Sventenia* Font Quer** LACTUCEAE

×*Prenanthenia* Svent., Addit. Fl. Canar. 1: 89 (1960) ‡

Natural hybrid:

P. pendula Sch.Bip. × *S. bupleuroides* Font Quer

×*P. rupicola* Svent., loc. cit. ‡

***Sventenia* Font Quer × *Taeckholmia* Boulos** LACTUCEAE

Natural hybrid:

Sventenia bupleuroides Font Quer × *Taeckholmia pinnata* (L.f.) Boulos (syn. *Sonchus leptocephalus* Sch.Bip.)

×*Sonchustenia decipiens* Svent., Addit. Fl. Canar. 1: 87 (1960) †

Note:

Sonchus leptocephalus Sch.Bip. is placed in *Taeckholmia* Boulos by Bremer (1994). To reflect this transfer, publication of a new condensed formula combining the names *Sventenia* and *Taeckholmia* is required.

2.3.2 Hybrids with nothotaxon names now usually classified as intrageneric and those initially misidentified as intergeneric hybrids

***Acosta* Adans. × *Calcitrapa* Hill** CARDUEAE

×*Acostitrapa* Rauschert, Feddes Repert. 83: 655 (1973) †⊕

Natural hybrids:

A. stoebe (L.) Soják × *C. solstitialis* (L.) Lam.

Centaurea hemiptera Borbás, Österr. Bot. Z. 28: 392 (1878) ‡

×*A. hemiptera* (Borbás) Rauschert, loc. cit. †

A. micranthos (Gmel.) Soják × *C. solstitialis* (L.) Lam.

Centaurea pseudohemiptera Wagner, Magyar Bot. Lapok. 17: 71 (1918) ‡

×*A. pseudohemiptera* (Wagner) Rauschert, loc. cit. †

A. diffusa (Lam.) Soják × *C. solstitialis* (L.) Lam.

Centaurea ×*subdiffusa*, Prodán, in Savul., Fl. Reipubl. Popul. Roman. 9: 951, 977 (1964) †⊕

×*A. subdiffusa* (Wagner) Rauschert, loc. cit. †

Note:

Both genera are included within *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

***Acosta* Adans. × *Colymbada* Hill** CARDUEAE

×*Colymbacosta* Rauschert, Feddes Repert. 83: 656 (1973) †⊕

Natural hybrid:

A. stoebe (L.) Soják × *C. scabiosa* (L.) Holub

Centaurea grabowskiana Wagner, Magyar Bot. Lapok. 15: 233 (1917), 'Grabowskyana' ‡

Centaurea ×*bubelae* Dostál, Kvetena CSR: 1689 (1950), nom. nud. ‡

×*Colymbacosta grabowskiana* (Wagner) Rauschert, Feddes Repert. 85: 653 (1974) †

?

Centaurea genesii-lopezii Fern.Casas & Susanna, Anales Jard. Bot. Madrid 39: ? (1982) ‡

×*Colymbacosta genesii-lopezii* (Fern.Casas & Susanna) Fern.Casas & Susanna, Fontqueria 2: 21 (1982) †

Notes:

Acosta Adans. and *Colymbada* Hill are included within *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

The putative parentage of ×*C. genesii-lopezii* is not given as I have not seen the original reference.

Acosta Adans. × _Jacea_ Mill. CARDUEAE

×*Jaceacosta* Rauschert, Feddes Repert. 83: 655 (1973) †⊕

Natural hybrids:

A. stoebe (L.) Soják × *J. pratensis* Lam.

Centaurea beckiana F. Müllner, Verh. Zool.-Bot. Ges. Wien 37: 27 (1888) ‡

×*Jaceacosta beckiana* (F. Müllner) Rauschert, loc. cit. †

A. micranthos (Gmel.) Soják × *J. pratensis* Lam.

Centaurea fortinata Wagner, Math. Természettud. Közlem. 30 (6): 130 (?) ‡

×*Jaceacosta fortinata* (Wagner) Rauschert, loc. cit. †

A. diffusa (Lam.) Soják × *J. pratensis* Lam.

Centaurea juvenalis Delile ex Godr., Mém. Acad. Montp. (sect. Médic.) 1: 433 (1853)‡

×*Jaceacosta juvenalis* (Delile ex Godr.) Rauschert, loc. cit. †

A. micranthos (Gmel.) Soják × *J. nigrescens* (Willd.)

Centaurea ×*pseudomicrantha* Rech.f., Österr. Bot. Z. 97: 121 (1950) ‡

×*Jaceacosta pseudomicrantha* (Rech.f.) Rauschert, loc. cit. †

A. stoebe (L.) Soják × *J. pratensis* Lam.

Centaurea teyberi Hayek, Denkschr. Kaiserl. Akad. Wiss., Wien, Math.-Naturwiss. Kl. 70: 675 (1901) ‡

×*Jaceacosta teyberi* (Hayek) Rauschert, loc. cit. †

Note:

Acosta Adans. and *Jacea* Mill. are included within *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

***Aetheopappus* Cass. × *Centaurea* L.** CARDUEAE

×*Centaureopappus* hort. ex Möllers, Deutsch. Gärtner-Zeit. 47: 166 (1932) ‡

Natural hybrid:

?

×*Centaureopappus hybridus* hort.

Notes:

Aetheopappus Cass. is included in *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

The putative parentage of this hybrid is not given as I have not seen the original reference.

***Anthemis* L. × *Chamomilla* S.F.Gray** ANTHEMIDEAE

×*Anthechamomilla* Rauschert, Fol. Geobot. Phytotax. 9: 258 (1974) †⊕

Natural hybrid:

A. cotula L. × *Chamomilla recutita* (L.) S.F.Gray (syn. *Matricaria recutita* L.)

Anthematricaria dominii Rohlena, Cas. Národ. Mus. 104: 9 (1930) †⊕

×*Anthechamomilla dominii* (Rohlena) Rauschert, Feddes Repert. 93: 10 (1983) †

Note:

Chamomilla S.F.Gray is recognised by Kay (in Tutin *et al.*, 1976) but is included within *Matricaria* L. by Bremer (1994), in which case the correct name for this hybrid is ×*Anthematricaria dominii* Rohlena (see p. 40).

***Anthemis* L. × *Cota* J.Gay ex Guss.** ANTHEMIDEAE

×*Cotanthemis* Smejkal ‡

Natural hybrids:

A. arvensis L. × *C. tinctoria* (L.) J.Gay

A. adulterina Wallr. ex E. Hallier, in Koch, Syn. Deutsch. Fl. Ed. 3: 1392 (1895) ‡

×*Cotanthemis adulterina* (Wallr.) Smejkal ‡

A. cotula L. × *C. tinctoria* (L.) J.Gay

A. bollei Asch. ex Nym., Consp. 2: 363 (?) ‡

×*Cotanthemis bollei* (Sch.Bip.) Smejkal ‡

A. austriaca Jacq. × *C. tinctoria* (L.) J.Gay

A. ochroleuca Celak.f. ex Ber., Deutsch. Bot. Ges. 5: 123 (1887) ‡

×*Cotanthemis ochroleuca* (Celak.f.) Smejkal ‡

Additional reference: Dostál (1982).

Notes:

Cota is usually considered a subgenus within *Anthemis* L. (e.g., Tutin *et al.*, 1976), in which case hybrids would be intrageneric.

Dostál (1982) listed the above nothotaxa but does not cite the reference in which Smejkal published new combinations, and I have been unable to locate this reference.

***Arctotis* L. × *Venidium* Less.** ARCTOTEAE

×*Venidioarctotis* hort. ⊕

Natural hybrids:

A. venusta Norl. × *V. fastuosum* Stapf. (syn. *A. fastuosa* Jacq.)

×*Venidioarctotis hybrida* hort.

A. venusta Norl. × *V. wyleyi* Harv. (syn. *A. wyleyi* Beauverd)

Additional references: Warren (1929); Everett (1982); Graf (1986); Huxley *et al.* (1992).

Note:

The condensed formula ×*Venidioarctotis* was applied to hybrids between species of *Venidium* Less. and *Arctotis* L. The *Venidium* species contributing to the hybrids are now included in *Arctotis* (Huxley *et al.*, 1992) and so the hybrids are now intrageneric.

The condensed formula ×*Venidioarctotis* is validly published. The earliest valid publication I am aware of is Graf (1986 p. 332), who used the orthography ×*Venidio-arctotis* and clearly indicates the name applied to *Arctotis* × *Venidium* hybrids. Everett (1982 p. 3476) discusses such hybrids but a multiplication symbol does not precede the condensed formula. Until a more exhaustive literature search is undertaken to identify the earliest valid publication of the name, the citation ×*Venidioarctotis* hort. is retained.

***Aster* L. × *Solidago* L.** ASTEREAE

×*Solidaster* H.R.Wehrh., in Bonstedt, Pareys Blumengärtn. 2: 525 (1932) ‡

×*Asterago* Everett, Gard. Chron. ser. III, 51: 6 (1937) †⊕

Natural hybrids:

A. ptarmicoides (Nees) Boivin × *S. canadensis* L.

×*Solidaster hybridus* H.R.Wehrh., loc. cit., nom. nud. ‡

×*Asterago lutea* Everett, loc. cit., nom. nud. ‡

×*Solidaster luteus* M.L.Green, Kew Bull. 1937: 352 (1937) ‡

Solidago ×*luteus* (M.L.Green) Brouillet & Semple, Can. J. Bot. 59: 21 (1981) †

A. ptarmicoides (Nees) Boivin × *S. ohioensis* Riddell

A. ptarmicoides (Nees) Boivin × *S. parvirigida* Beaudry

A. ptarmicoides (Nees) Boivin × *S. riddellii* Frank

Additional references: Arends (1931); Bernard (1969).

Notes:

Natural hybrids between *Aster ptarmicoides* and four *Solidago* species are recorded (Arends, 1931; Bernard, 1969), but Brouillet and Semple (1981) concluded *A. ptarmicoides* was best placed in *Solidago* sect. *Oligoneuron* and so these hybrids have been considered intrageneric. Some authors (e.g., Nesom, 1993) recognise *Oligoneuron* Small as a distinct genus, in which case these hybrids would be intergeneric and publication of a new condensed formula combining the names *Solidago* and *Oligoneuron* would be required.

×*Solidaster* H.R.Wehrh. is the earliest validly published name for hybrids between *Aster* and *Solidaster*, thus ×*Asterago* Everett is a synonym. Powell (1985) used the name "*Solidigaster*" but it was not preceded by a multiplication symbol and so was not validly published. I am not aware of this name being used in any other publication.

***Boltonia* L'Hér. × *Chrysanthemum* L.** ANTHEMIDEAE

×*Chrysaboltonia* Arends, Preis-Verzeichn. 1936-7: 6 ‡, & Haupt-Verzeichn. 1938: 7 ‡; Heydenr., Gartenschönh. 19: 55 (1938) ‡; cf. Sealy, Curtis's Bot. Mag. 161:sub. t. 9566 (1939) †

Natural hybrid:

?

×*Chrysaboltonia pulcherrima* Arends; Heydenreich; Sealy in syn.

Chrysanthemum rubellum Sealy, J. Roy. Hort. Soc. 63: 266 (1938) ‡

Dendranthema ×*rubellum* (Sealy) Philp, Atlas Kent Fl.: 140 (1982) ‡

Dendranthema zawadskii (Herbich) Tzvelev

Additional references: Sealy (1939); Huxley *et al.* (1992).

Notes:

Although initially considered a putative hybrid between species of *Boltonia* and *Chrysanthemum*, it is now considered to be attributable to *Dendranthema zawadskii*. Sealy (1939) discussed what was known about its history. He noted that *Boltonia* and *Chrysanthemum sensu lato* belong to separate tribes (*Boltonia* to the Astereae, *Chrysanthemum* to the Anthemideae) and therefore it would be highly unlikely the two genera are capable of hybridising, although intertribal hybrids are known in the Gramineae (Stace, 1975). Sealy also stated there was no indication of *Boltonia latisquama* (one of the putative parents) in its morphology. He considered it a typical member of the Pyrethrum group in *Chrysanthemum sensu lato* and published the name *Chrysanthemum rubellum*. It has since been transferred to *Dendranthema* following recircumscription of *Chrysanthemum* L. and included within *D. zawadskii* (see Huxley *et al.*, 1992). The ultimate origin of the name ×*Chrysaboltonia* remains unknown.

***Calcitrapa* Hill × *Colymbada* Hill** CARDUEAE

×*Calcitrymbada* Smejkal ‡

Natural hybrid:

Calcitrapa soltitialis (L.) Lam. × *Colymbada scabiosa* (L.) Holub

Centaurea trautmannii Wagner, Magyar. Bot. Lapok. 19: 31 (1922) ‡

× *Calcitrymbada trautmannii* (Wagner) Smejkal ‡

Additional reference: Dostál (1982).

Notes:

Both genera are included within *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

Dostál (1982) listed the above nothospecies but did not cite the reference in which the names were published, and I have been unable to locate this reference.

***Calcitrapa* Hill × *Jacea* Mill.** CARDUEAE

×*Jaceitrapa* Rauschert, Feddes Repert. 83: 656 (1973) †⊕

Natural hybrids:

C. solstitialis (L.) Lam. × *J. pratensis* Lam.

Centaurea amphibola Hausskn., Mitt. Geogr. Ges. (Thüringen) Jena 3: 224, 228 (1885) ‡

×*Jaceitrapa amphibola* (Hausskn.) Rauschert, loc. cit. †

C. stellata Lam. × *J. pseudophrygia* (C.A.Mey.) Holub

Centaurea reditus F.Herm., Repert. Spec. Nov. Regni Veg. 17: 449 (1921) ‡

×*Jaceitrapa reditus* (F.Herm.) Rauschert, Feddes Repert. 85: 654 (1974) †

Note:

Calcitrapa Hill and *Jacea* Mill. are usually included within *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

***Chamomilla* S.F.Gray × *Matricaria* L.** ANTHEMIDEAE

×*Matrichamomilla* Rauschert, Feddes Repert. 83: 655 (1973) †⊕

Notes:

Rauschert listed no collective epithets or hybrid formulas for ×*Matrichamomilla* and I am unaware of any other published references to such hybrids. Article 34.1(b) of the *International Code of Botanical Nomenclature* (Greuter *et al.*, 2000) states, "names published merely in anticipation of the existence of a hybrid are not validly published", thus the name ×*Matrichamomilla* might not be validly published.

Chamomilla S.F.Gray is recognised by Kay (in Tutin *et al.*, 1976) but is included within *Matricaria* L. by Bremer (1994), in which case hybrids would be intrageneric.

***Colymbada* Hill × *Jacea* Mill.** CARDUEAE

×*Colycea* Fern.Casas & Susanna, Fontqueria 2: 21 (1982) †⊕

Natural hybrid:

?

Centaurea valdesii-bermejoi Fern.Casas & Susanna, Anales Jard. Bot. Madrid 39: ? (1982) ‡

×*Colycea valdesii-bermejoi* (Fern.Casas & Susanna) Fern.Casas & Susanna, loc. cit.†

Notes:

Colymbada Hill and *Jacea* Mill. are included within *Centaurea* L. *sensu lato* by Bremer (1994), in which case hybrids would be intrageneric.

The putative parentage of ×*C. valdesii-bermejoi* is not given as I have not seen the original reference.

***Crepidiastrum* Nakai × *Paraixeris* Nakai** LACTUCEAE

×*Crepidiastrixeris* Kitam., Acta Phytotax. Geob. 6: 235 (1937) †⊗

Natural hybrids:

C. keiskeanum (Maxim.) Nakai × *P. denticulata* (Houtt.) Nakai

Paraixeris surugensis Hisauchi, J. Jap. Bot. 10: 697 (1934) ‡

Crepidiastrixeris surugensis (Hisauti) Kitam., Acta Phytotax. Geob. 6: 236 (1937) †

C. lanceolatum (Houtt.) Nakai × *P. denticulata* (Houtt.) Nakai

Crepidiastrixeris denticulatolanceolata Kitam., Acta Phytotax. Geob. 11: 132 (1942) †⊗

C. platyphyllum (Franch. & Savat.) Kitam. × *P. denticulata* (Houtt.) Nakai

Lactuca denticulatoplatyphylla Makino, J. Jap. Bot. 1: 11 (1917) ‡⊗

Paraixeris denticulatoplatyphylla (Makino) Nakai, Bot. Mag. Tokyo 34: 157 (1920) ‡

Crepidiastrixeris denticulatoplatyphylla (Makino) Kitam., loc. cit. †

Additional references: Ono and Sato (1935); Ono (1941, 1946, 1950, 1951, 1955); Ohwi (1965); Pak and Kawano (1992); Iwatsuki *et al.* (1993).

Notes:

The condensed formula ×*Crepidiastrixeris* was not validly published by Kitamura (1937), as it was not preceded by a multiplication symbol, but it is validly published elsewhere (e.g., Iwatsuki *et al.*, 1993). Thus a literature search is required to determine the earliest valid publication of this name.

In accordance with the current botanical code, the names '*denticulatoplatyphylla*' and '*denticulatolanceolata*' are formulas rather than epithets, hence publication of new collective epithets is required.

Opinions on the correct placement of the parental species vary widely. *Paraixeris* is recognised by Iwatsuki *et al.* (1993) but not by Bremer (1994), Ohwi (1965) included *Paraixeris denticulata* in *Youngia*, and Pak and Kawano (1992) reduced *Paraixeris* to a section within *Crepidiastrum*.

***Crepis* L. × *Hieracium* L.** LACTUCEAE

Crepi-Hieracium P.Fourn., Fl. Compl. Plaine Franç.: 316 (1928) †⊗

Natural hybrid:

C. praemorsa L. × *H. murorum* Petitm.

Crepis garnieri Petitm., Bull. Soc. Sci. Nancy ser. 3, 8: 213 (1906) ‡

Crepi-Hieracium garnieri (Petitm.) P.Fourn., loc. cit. †

Notes:

The validity of this hybrid is uncertain. The putative parentage follows Fournier (1928), whom in a later flora (Fournier, 1940) makes no mention of this hybrid. Babcock (1947) lists it as an intrageneric hybrid (as "× *Crepis garnieri*") but could not locate the original reference and made no comment on its validity or parentage. I am unaware of any reference to it in more recent publications.

The two genera are placed in separate subtribes by Bremer (1994).

The nothogenus '×*Crepihieracium*' was not validly published by Fournier (1928), as it was not preceded by a multiplication symbol and to my knowledge has not been validly published by another author.

***Dubautia* Gaudich. × *Railliardia* Gaudich.** HELIANTHEAE

×*Railliautia* Sherff, Bernice P. Bishop Mus. Bull. 135: 136 (1935) †⊕

Natural hybrids:

D. plantaginea Gaudich. × *R. scabra* DC.

D. ×*fallax* Sherff, Bot. Gaz. 96: 150 (1934) †⊕

D. ×*fucosa* Sherff, loc. cit. 149 †⊕

×*Railliautia fallax* (Sherff) Sherff, loc. cit. †

×*R. fucosa* (Sherff) Sherff, loc. cit. †

Additional reference: Carr (1990).

Note:

Railliardia is now regarded as a section within *Dubautia* (Carr, 1985), in which case hybrids would be intrageneric.

***Leontodon* L. × *Scorzoneroides* Moench** LACTUCEAE

×*Leontoroides* Dostál, Seznam cévnatych rostlin kveteny ceskoslovenské: 286 (1982) †⊕

Natural hybrid:

L. incanus Schrank × *S. autumnalis* (L.) Moench

Leontodon ×*hispidaster* Beauverd, Bull. Soc. Bot. Genève, ser. 2, 12: 153 (1921) ‡

L. ×*karpatianus* Soó, Acta Bot. Acad. Sci. Hung. 1: 227 (1954), sine descr. ‡

L. ×*ambiguus* Fleisch. ‡

×*Leontoroides hispidaster* (Beauverd) Dostál, loc. cit. †

Note:

Scorzoneroides Moench is usually considered a section within *Leontodon* L. (e.g., Tutin *et al.*, 1976), in which case hybrids would be intrageneric.

2.3.3 Artificial and putative natural intergeneric combinations lacking nothogeneric names

The generic concepts of Bremer (1994) were again followed when compiling Table 2., with the following exceptions: the concepts of *Ewartia*, *Raoulia*, and the New Zealand taxa currently retained in *Helichrysum* follows Ward and Breitwieser (1998a). The generic concepts of Baldwin (1999) are not included.

Cross	Tribe	Type	References
Achillea × *Anthemis*	Anthemideae	N	Fiori (1969)
Ajania × *Dendranthema*	Anthemideae	E	Boase *et al.* (1997)
Anaphalioides × *Ewartia*	Gnaphalieae	N	Allan (1961)
Anaphalioides × *Helichrysum*	Gnaphalieae	N	Heenan (1989); Jordan (1995)
Anaphalioides × *Leucogenes*	Gnaphalieae	N	Falvey (1996)
Anaphalioides × *Raoulia*	Gnaphalieae	N	Falvey (1996)
Anthemis × *Chamaemelum*	Anthemideae	E	Mitsuoka and Ehrendorfer (1972)
Arctanthemum × *Dendranthema*	Anthemideae	E	Boase *et al.* (1997)
Argyroxiphium × *Wilkesia*	Heliantheae	E	Kyhos *et al.* (1990)
Aster × *Heteropappus*	Astereae	?	Knobloch (1972)
Aster × *Kalimeris*	Astereae	N	Tara (1979)
Aster × *Machaeranthera*	Astereae	E	Stucky (1978); Nesom (1994)
Chrysanthemum × *Ismelia*	Anthemideae	E	Chaudhuri *et al.* (1976)
Chrysopsis × *Heterotheca*	Astereae	E	Harms (1965); Knobloch (1972)
Chrysothamnus × *Haplopappus*	Astereae	N	Anderson and Reveal (1966)
Crepidiastrum × *Lactuca*	Lactuceae	?	Ono (1955)
Crepis × *Taraxacum* *	Lactuceae	N/E	Sinotô & Ono (1934); Stace (1975)
Damnamenia × *Pleurophyllum*	Astereae	N	Given (1973)
Dendranthema × *Tanacetum*	Anthemideae	E	Kondo *et al.* (1999)
Dubautia × *Madia*	Heliantheae	E	Baldwin (1996)
Dubautia × *Madia* × *Raillardiopsis*	Heliantheae	E	Carr *et al.* (1996)
Dubautia × *Raillardiopsis*	Heliantheae	E	Kyhos *et al.* (1990)

Table 2.2. Experimental and putative natural intergeneric hybrid combinations lacking nothogeneric names. E, experimental hybrids; N, natural hybrids; *, of doubtful authenticity; ?, unknown. (continued overleaf)

Table 2.2 (continued).

Cross	Tribe	Type	References
Dubautia × *Wilkesia*	Heliantheae	E	Kyhos *et al.* (1990)
Encelia × *Geraea*	Heliantheae	N	Kyhos (1967)
Euchiton × *Leucogenes*	Gnaphalieae	N	Drury (1972)
Filago × *Gnaphalium* *	Gnaphalieae	N	Stace (1975)
Flaveria × *Haploësthes*	Helenieae	E	Powell (1978)
Flaveria × *Sartwellia*	Helenieae	E	Powell (1978)
Grindelia × *Haplopappus*	Astereae	E	Nesom (1994)
Haploësthes × *Sartwellia*	Helenieae	E	Powell (1978)
Haplopappus × *Isocoma*	Astereae	E	Jackson and Dimas (1981)
Helianthus × *Tithonia*	Heliantheae	E	Christov and Panayotov (1991)
Helianthus × *Viguiera*	Heliantheae	E	Heiser (1963)
Helichrysum × *Leucogenes*	Gnaphalieae	N	Allan (1961); Molloy (1980)
Helichrysum × *Raoulia*	Gnaphalieae	N	Brockie (1956); Allan (1961)
Helianthus × *Verbesina*	Heliantheae	E	Vassilevska-Ivanova *et al.* (1996)
Hemizonia × *Holozonia*	Heliantheae	E	Clausen *et al.* (1937)
Heteropappus × *Kalimeris*	Astereae	N/E	Huziwara (1950); Inoue (1961)
Isocoma × *Xanthocephalum*	Astereae	N	Hartman and Lane (1991)
Koanophyllon × *Pseudokyrsteniopsis*	Eupatorieae	E	Powell (1985)
Lactuca × *Paraixeris*	Lactuceae	N/E	Ono (1943); Ono and Sakai (1952); Ono and Nagai (1958)
Layia × *Madia*	Heliantheae	E	Clausen *et al.* (1937)
Leucanthemella × *Nipponanthemum*	Anthemideae	E	Ogura and Kondo (1998)
Linosyris × *Galatella*	Astereae	N	Nesom (1994)
Lipochaeta × *Wedelia*	Heliantheae	E	Rabakonandrianina & Carr (1981)
Madia × *Raillardiopsis*	Heliantheae	E	Kyhos *et al.* (1990)
Matricaria × *Pentzia*	Anthemideae	E	Mitsuoka and Ehrendorfer (1972)
Picris × *Sonchus*	Lactuceae	N	Ono and Sakai (1954)
Raillardella × *Raillardiopsis*	Heliantheae	E	Kyhos *et al.* (1990)
Rigiopappus × *Tracyina*	Astereae	E	Ornduff (1975)

2.4 Discussion

The above lists contain a total of 70 intergeneric combinations distributed among ten tribes (Table 2.3 p. 61). It is possible other natural intergeneric hybrids occur but have yet to be detected or reported. There may be other published references of which I am unaware and experimental crosses not yet attempted between related genera lacking the opportunity to

Tribe	Number of intergeneric combinations		
	Natural	Solely experimental	Total
Anthemideae	6	7	13
Astereae	10	6?	16
Cardueae	3	0	3
Eupatorieae	0	1	1
Gnaphalieae	9	0	9
Helenieae	1	3	4
Heliantheae	2	13	15
Lactuceae	7?	1?	8
Mutisieae	1	0	1
Senecioneae	1	0	1
TOTAL	40	31	71

Table 2.3. Number of natural and experimental intergeneric combinations per Compositae tribe, collated from the lists presented in section 2.2. Crosses between different species of the same genera are not included in the figures.

hybridise naturally may also greatly expand the range of successful crosses. Thus the lists may underestimate the extent and potential for intergeneric hybridisation in the Compositae. The crosses reported have been substantiated to varying degrees; some may be erroneous, but determination of the authenticity of the reported hybrids was not within the scope of this literature review.

It is difficult to produce a definitive list of intergeneric hybrids in the Compositae until opinions on generic concepts stabilise. As an example, acceptance of segregate genera of *Centaurea* (see Bremer, 1994 p. 125–128) would result in the recognition of up to seven additional intergeneric combinations. One outcome of the splitting of *Centaurea* has been the publication of six nothogenera to accommodate nothospecies with collective epithets. However, the segregate genera have not yet gained general acceptance (as in Bremer, 1994). The status of several other nothotaxa (e.g., ×*Crepidiastrixeris*, ×*Heterokalimeris* and ×*Macropertya*), here classified as intergeneric, will differ depending on the generic concepts followed. The redefinition of *Chrysanthemum* into more than 30 segregate genera has resulted in five intergeneric hybrid combinations. Divergent opinions on generic concepts also impacts on the number of intergeneric hybrids involving *Aster* and related genera.

Of the hybrids listed by Knobloch (1972), ×*Asterago* is a synonym for ×*Solidaster*, the parental genera have been amalgamated in three instances (*Arctotis* × *Venidium*, ×*Centaureopappus* and ×*Railliautia*), at least one was misidentified or erroneous (×*Chrysaboltonia* and probably *Crepi-Hieracium garnieri*) and several nothogenera currently lack recorded hybrids as a result of changes in generic concepts (×*Anthechrysanthemum*, ×*Chrysanthemoachillea*, ×*Leucopyrethrum*, ×*Senecillicacalia* and ×*Solidaster*). Knobloch (1972) did not distinguish between natural and experimental crosses in his list of intergeneric hybrids, but Stace (1975, p. 13) stated that over half of Knobloch's complete list were solely artificial crosses. In the present lists for the Compositae, about two-thirds are natural crosses. Several factors may account for this difference: the high number of artificial crosses in the Gramineae and Orchidaceae, which together made up about 90% of Knobloch's list; the relative difficulty of performing artificial crosses between members of the Compositae (for example, the florets are small and difficult to manipulate); or a lower level of interest relative to families of greater horticultural (e.g., Orchidaceae) or agricultural (e.g., Gramineae) importance.

The Astereae contains the highest frequency of intergeneric combinations, but this may simply reflect a greater intensity of study rather than a greater propensity to hybridise. Eight of the nine intergeneric combinations in the Gnaphalieae occur between New Zealand taxa. Hybrids between *Filago vulgaris* and *Logfia arvensis* appear to be the only recorded Gnaphalieae intergeneric hybrids elsewhere. The cross *Filago gallica* × *Gnaphalium uliginosum* has been reported from France but is of doubtful authenticity (Stace, 1975). More intensive study and experimental crosses between taxa from other countries might result in other successful crosses in the Gnaphalieae.

Stace (1975 p. 93) noted, "Our state of knowledge concerning the nomenclature of hybrids is far less advanced than that concerning species" and accepted some nothotaxon names he listed may need to be rejected or replaced in the future. The above survey indicates numerous nothotaxon names were not validly published by certain authors, such as Kitamura and Domin, and there is a relatively high level of synonymy, reflecting changes in generic concepts or incorrect determination of parentage. Recent changes in generic concepts means that publication of new condensed formulas, combining the names of the putative parental genera, are required for the following combinations: *Achillea* × *Tanacetum*, *Filago* × *Logfia*, *Ligularia* × *Parasenecio* and *Sventenia* × *Taeckholmia*. As a result, the nothogenera

×*Chrysanthemoachillea*, ×*Giflifa*, ×*Senecillicacalia* and ×*Sonchustenia* currently do not contain any recorded nothotaxa. Publication of a new condensed formula for hybrids between *Matricaria* and *Tripleurospermum* is required as ×*Pseudomatricaria* does not comply with the Botanical Code. New combinations for nothotaxa in ×*Anthepleurospermum*, ×*Carduocirsium* and ×*Leucoraoulia* are required, and new collective epithets are required for two nothotaxa in ×*Crepidiastrixeris*. Several putative intergeneric hybrids were originally described as species but currently lack nothogeneric names. For nomenclatural clarity these ideally should be given nothogeneric names once generic concepts stabilise. In addition to the indigenous hybrids listed in Table 2.2 (pp. 59–60), *Achillea hausmanniana* Suenderm. was deemed by Fiori (1969) to represent hybrids between *Achillea clavenae* L. and *Anthemis alpina* L.

The cross *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill.) Thell. × *Taraxacum officinale* Weber agg. was reported from Worcestershire, England without substantive evidence in 1908 but Stace (1975) dismissed it as extremely unlikely. Putative hybrids between *Crepis capillaris* (L.) Wallr. and *Taraxacum platycarpum* Dahlst. were raised by Sinotô and Ono (1934), but the evidence presented did not conclusively demonstrate the plants' hybrid origin. Abnormally developed leaves were described, but the putative hybrids possessed the same chromosome number as the maternal parent (either $2n = 6$ or $2n = 16$) rather than an intermediate level. Thus, on current evidence, reports of hybrids between *Crepis* and *Taraxacum* are of doubtful validity.

This survey indicates the existence within the Compositae of several groups of closely related genera that, despite morphological and ecological divergence, retain the capacity to hybridise. The New Zealand Gnaphalieae have a propensity to hybridise, with hybrids between *Anaphalioides*, *Euchiton*, *Ewartia*, *Helichrysum*, *Leucogenes* and *Raoulia* reported in the literature (Brockie, 1956; Allan, 1961; Drury, 1972; Molloy, 1980; Jordan, 1995; Falvey, 1996). In the Anthemideae, cytogenetic data and cross-compatibility indicates the genera *Anthemis*, *Chamaemelum*, *Matricaria* and *Tripleurospermum* are closely related (Mitsuoka and Ehrendorfer, 1972) and recent molecular evidence suggests *Tanacetum* is also close to members of this group (Oberprieler and Vogt, 2000). Reports of hybrids linking *Achillea*, *Ajania*, *Arctanthemum*, *Chrysanthemum*, *Dendranthema*, *Ismelia*, *Leucanthemum* and *Tanacetum* further extends the web of relationships. The reputed cross-compatibility of *Leucanthemum vulgare* and *Tanacetum corymbosum* conflicts with recent cladistic and molecular genetic studies, which suggest the species are not closely related (Bremer and

Humphries, 1993; Oberprieler and Vogt, 2000). The Hawaiian silversword alliance (Heliantheae: Madiinae) comprise another interfertile group of genera comprising *Argyroxiphium*, *Dubautia* and *Wilkesia*, and experimental hybrids with North American *Madia* and *Raillardiopsis* species have been synthesised (Kyhos *et al.*, 1990; Carr *et al.*, 1996). Japanese Lactuceae are another example, with hybrids between *Crepidiastrum*, *Ixeris*, *Lactuca*, *Lapsana*, *Paraixeris* and *Youngia* reported (Kitamura, 1942; Ono, 1951; Ono and Sakai, 1952; Ono, 1955; Ono and Nagai, 1958; Iwatsuki *et al.*, 1993).

Thus, existence of a group of closely related genera, which although morphologically and ecologically diverse are still capable of producing viable hybrids, is a feature of several tribes in the Compositae. In this regard, the Compositae appears to be similar to the Aspleniaceae, Gramineae and Orchidaceae, which are actively evolving, often with poorly defined generic boundaries, and in which intergeneric hybrids are unusually frequent (see Stace, 1975).

SECTION TWO

Case studies of natural putative intergeneric hybrids in the New Zealand Gnaphalieae

Chapter 3. Recognition of hybrids and character descriptions

3.1 Recognition of hybrids

Stace (1975, 1989) discussed five criteria important for the recognition of hybrids: phenetic intermediacy between the putative parental species, field evidence, segregation in the F_2 generation, reduced fertility and experimental synthesis. Additional criteria have been deemed important by other authors. Gottlieb (1972), for example, also included: an additive profile for biochemical characters present in each parental species but not both; occurrence on more recent geological formations than the parental species; and intermediacy for physiological characters. Fulfilment of as many criteria as possible is desirable, but no generalisations or set of rules can be applied in all instances. For example, the putative hybrids may be absolutely sterile and so production of an F_2 generation is impossible. The more criteria that are fulfilled, the greater the confidence in the hybridity hypothesis (Gottlieb, 1972).

Experimental resynthesis of hybrids by crossing the putative parental species and comparison of the experimental and natural hybrids provides perhaps the most conclusive evidence for hybridity. Baker (1947) advocated the need to perform artificial crosses before embarking on a detailed morphological study of putative hybrids. However, there are many instances where experimental attempts to recreate natural hybrids of known parentage have been unsuccessful, or the experimental hybrids may differ from the natural hybrids due to differences in the parental genotypes (Stace, 1989). Such instances are not necessarily evidence against a hybridity hypothesis.

In hybrids individual characters may be either intermediate between the parental states, identical to one or both parents, more extreme than the parental states, or novel (Rieseberg and Ellstrand, 1993; McDade, 1995). Hybrids are generally expected to exhibit *overall* intermediacy between their parents, but genetic and environmental factors might produce non-intermediate hybrids (McDade, 1997), especially among later-generation hybrids in which extreme and novel characters are often more frequent (Rieseberg and Ellstrand, 1993). *Intermediacy in individual characters* is evidence for hybridity, but a *mixture of parental character states* could be a result of ancestral hybridity or divergence (Wilson, 1992). Rieseberg and Ellstrand (1993) surveyed the expression of different types of markers in hybrids and concluded chemical and molecular characters are usually intermediate in F_1

hybrids and hybrid taxa, whereas morphological characters often behave less predictably and are less reliable for diagnosing hybrids. Although matrocliny, heterosis and dominance effects can affect character expression, "wholesale rejection of morphological characters for hybrid identification due to the unpredictability of hybrid character expression is unwarranted" (Estabrook *et al.*, 1996 p. 650). Similarly, chemical expression can also vary among hybrids. Hybrids may express all or only some parental chemicals or produce novel compounds, and concentrations produced can vary markedly depending on the parental taxa, hybrid class (i.e., F_1 or later generation and backcrosses), ploidy level, chemical class and the genetics of inheritance (e.g., the presence of dominance or additive inheritance) (Orians, 2000). Therefore consideration of one or two character types only might lead to erroneous hypotheses of hybridity or misclassification of hybrids (Wilson, 1992; Rieseberg and Linder, 1999). As an example, from morphological and geographical evidence hybrids between *Lepidothamnus laxifolius* (Hook.f.) Quinn and *L. intermedius* (Kirk) Quinn were for many years thought to be *L. laxifolius* × *Halocarpus bidwillii* (Kirk) Quinn hybrids, but analysis of their chemistry and chromosome number conclusively identified the parental species (Quinn and Rattenbury, 1972). In the New Zealand Gnaphalieae, *Rachelia glaria* and *Helichrysum dimorphum* Cockayne are species that, on account of their morphology and localised distributions, have been suggested erroneously to be intergeneric hybrids (Wall, 1920; Allan, 1961).

Numerous analytic methods have been used to aid identification of hybrids. Hybrid indices, character counts and pictorialised scatter diagrams are the simplest techniques and often are just as effective as more sophisticated techniques (Goodman, 1967; Wilson, 1992). In the quest for greater objectivity, multivariate analytic methods, such as cluster analysis, multiple discriminant analysis and multidimensional scaling, have been utilised (e.g., Pimentel, 1981; Adams, 1982; Hollingsworth *et al.*, 1998; Brochmann *et al.*, 2000). In addition, the HYWIN computer program was developed specifically for hybrid identification (Estabrook *et al.*, 1996).

Plant distributions provide some of the most useful circumstantial evidence for identifying hybrids (Stace, 1989). The proximity of species to the putative hybrids and ecological and geographical evidence can be valuable. Usually one or both of the putative parental species grow in the vicinity of the putative hybrids. In some instances, hybrids occur in ecologically intermediate habitats (e.g., Cruzan and Arnold, 1993). However, exceptions may occur as a result of long-distance dispersal of pollen or seeds, the disappearance of one or both parents from a locality, or the spread of a hybrid away from the original locality (Stace, 1989).

Analysis of character segregation in F_2 progeny may be informative where more direct methods of investigation are unavailable (Stace, 1975). The degree of variation amongst F_1 hybrids will depend on the heterozygosity of the parents and genetic regulation of the phenotype, but genetic recombination in the F_2 generation usually results in a wide range of variation between the parental extremes and new combinations of parental traits, as illustrated by the leaf morphology of F_2 hybrids between *Bidens ctenophylla* Sherff and *B. menziesii* Sherff (Mensch and Gillett, 1972) and a hybrid swarm between *Argyranthemum broussonetii* (Pers.) C.J.Humphries and *A. frutescens* (L.) Schultz Bip. (Brochmann, 1987). Character segregation in the progeny of an individual suspected of being an F_1 hybrid may be evidence of hybridity, but should be interpreted with caution. The absence of segregation in a trait may be due to dominance effects or the plant may be a later-generation hybrid with lower heterozygosity.

Although many hybrids possess reduced fertility, this is not an absolute criterion of hybridity (Stace, 1975). A continuum between absolute sterility and full fertility exists and the degree of fertility is not correlated with the taxonomic distance between the parents. For example, some intergeneric hybrids are partially fertile (e.g., Mitsuoka and Ehrendorfer, 1972; Kyhos *et al.*, 1990), but *Spartina* ×*townsendii* Groves & J.Groves is an example of an absolutely sterile interspecific hybrid (Stace, 1975). Stace (1989) suggested most hybrids that attain maturity are fertile to some degree and concluded that few hybrids have been demonstrated to be absolutely sterile. Reduced fertility in hybrids may be attributable to various meiotic abnormalities, such as unpaired chromosomes (univalents), multiple chromosome pairings (multivalents), chromosome breakage, spindle abnormalities, chromosome bridges, misdivision of centromeres and degeneration of the post-meiotic sporocytes. However, the level of meiotic pairing and fertility are not always correlated and pairing between homologous chromosomes may be genetically suppressed in hybrids (see John and Lewis, 1965). Hybrids between species differentiated primarily by genic factors tend to have regular meiotic pairing and high fertility, whereas in hybrids between species with numerical or structural differences in chromosomes, pairing tends to be irregular and fertility is usually reduced (John and Lewis, 1965).

3.2 Types of characters

A taxonomic character is "any attribute of a member of a taxon by which it differs or may differ from a member of a different taxon" (Mayr, 1969). All taxa are therefore distinguished by their possession of unique characters or combinations of characters. Continuous characters

include absolute measures and ratios, for which no gap exists between two potentially observable values. Discrete characters are expressed as classes, which can be ordered multistate, non-ordered multistate or binary. Counts are, strictly speaking, discrete characters. However, it is sometimes more useful to treat discrete characters as continuous variables when the possible values are close together and cover a wide range of numbers (Kleinbaum *et al.*, 1987 section 2-1-1). The coding of discrete characters with three or more states is a common problem; they can be treated as a single multistate character or binary characters, but the latter leads to the problem of inapplicable characters or unequal weighting (Seitz *et al.*, 2000).

Because character states in hybrids are often intermediate between the parents, in hybridity studies the most informative characters are often those allowing clear discrimination of species and of an intermediate state. Intermediate characters may occur in states that form a continuum between the parental states or in discrete classes and so both continuous and discrete characters can indicate hybrid intermediacy. Many taxonomic descriptive terms conceal variation and so are less useful for detecting hybridity unless the parental species differ widely. Inclusion of characters that vary independently of hybridity or introgression adds 'noise', disrupting underlying patterns that may exist and making interpretation more difficult (e.g., Hatheway, 1962). Novel characters are more frequent in later-generation hybrids (Rieseberg and Ellstrand, 1993) but are of no value for identifying hybrids. Parental characters, particularly those in which the character state is unique to a species, can also provide strong evidence for hybridity.

3.3 Data analysis

No single method of analysis has yet been developed that has been proved to identify hybrids reliably for all data types. Numerous multivariate methods has been utilised to analyse hybridity data including: character counts, hybrid indices, discriminant analysis, cluster analysis, multidimensional scaling, minimal spanning trees, detrended correspondence analysis, split decomposition and the HYWIN computer program. These methods vary in their assumptions, utilise different types of input data and differ in their means of estimating or representing relationships. Some methods operate on the raw data, but some require estimates of resemblance (distances, similarities or dissimilarities) to be calculated from the original data. The interpretability or validation of the results is problematic for some techniques. In addition, their ability to characterise hybrids from the same data can vary (e.g., Pimentel, 1981; Adams, 1982; Brochmann, 1987; Wilson, 1992; McDade, 1997). Consequently, a

multiple-method approach is usually employed in hybridity studies to overcome these problems. Methods that place hybrids predictably are the most useful for identifying hybrids. Intermediacy between the parents is often considered a criterion for recognising hybrids, but placement of the putative hybrids with one or both parents can also be informative, particularly if a hybridity hypothesis exists, and genetic and environmental factors can result in non-intermediacy of some hybrids (McDade, 1997).

Character counts have been used previously in studies of intergeneric hybrids (Bateman and Farrington, 1987; Hawkins *et al.*, 1999). This simple method quantifies the proportion of character states in a putative hybrid that are intermediate or non-intermediate between the hypothesised parental species. Bateman and Farrington (1987) classified continuous characters in putative hybrids as 'intermediate', 'equivocal' or 'extreme'. Intermediacy was determined by comparing the mean in the putative hybrid with the standard deviation intervals (the mean ± the standard deviation) of the putative parental species. A preponderance of intermediate character states provides evidence for hybridity over hybrid speciation or divergence (Wilson, 1992).

A hybrid index, in its original form (Anderson, 1949), involved the scoring of continuous characters as discrete classes and transformation of characters so that one putative parent always received the lowest score and the other putative parent received the highest score. The sum of an individual plant's character scores represented its hybrid index. Because satisfactory definition and delimitation of the classes for each character may be difficult and result in illogical groupings or loss of information (Brochmann, 1987), various modifications have been proposed (Hatheway, 1962; Namkoong, 1966; Goodman, 1967; Brochmann, 1987). Nevertheless, simple hybrid indices have been utilised in recent studies of hybrids (e.g., Dafni and Baumann, 1982; Hodálová and Marhold, 1996).

Discriminant analysis is often used in hybridity studies (e.g., Brochmann *et al.*, 2000). There are several methods depending on the nature of the data and the objectives (see Huberty, 1994). Linear discriminant analysis generates linear combinations of the characters, which are weighted in order to achieve the best discrimination of predetermined groups. Canonical discriminant analysis is a form that seeks to minimise the number of dimensions required to depict relationships. Each combination of transformed variables is termed a 'linear discriminant' or 'canonical variate' and maximises the between-group variance relative to the within-group variance. The relative contribution of each character to each discriminant

function can be examined and group membership can be predicted for a given plant. Quadratic discrimination is superior with increasing inequality of group-covariance matrices (i.e., heteroscedasticity) and decreasing group separation, but if the ratio of groups to characters is small, a linear classification rule is preferable, even with covariance inequality (see McLachlan, 1992 p. 133). A linear rule provides "potential for greater across-sample stability of results (with or without normality)" (Huberty, 1994 p. 64).

Numerous coefficients for estimating resemblance between taxa or individuals have been published (Mardia *et al.*, 1979 pp. 375-381; Sneath and Sokal, 1973 pp. 116-145). Gower's (1971) general coefficient of similarity, which incorporates three separate coefficients, is often used in hybridity studies (e.g., Pimentel, 1981; Brochmann, 1987). It has the advantages of tolerating missing data and enabling the use of 'mixed' data sets (i.e., containing both continuous and discrete characters). The distances can be analysed by a number of methods, of whioch clustering and ordiunation methods are commonly utilised in hybridity studies. However, McDade (1997) concluded that for recognition of hybrids, identification of the taxa most similar to the putative hybrids from the raw distance values was more informative than analytic methods operating on the distances.

Cluster analysis partitions phenetically similar OTUs in a single dimension and emphasises discontinuities rather than continuous variation. Clustering procedures can be divided into hierarchical and partitioning methods (see Kaufman and Rousseeuw, 1990). Hierarchical clustering seeks to represent dissimilarities in a nested, non-overlapping, hierarchical structure. A number of linkage methods can be employed. 'Group-average' (or UPGMA) linkage is the most commonly used and utilises the average dissimilarity between the OTUs of each cluster. Partitioning methods assign the OTUs to one of a predetermined number of clusters. Partitioning around medoids (or PAM clustering) seeks to produce 'spherical' clusters centred on a representative OTU (or medoid) for each cluster, whereas fuzzy partitioning is less restrictive and allows for differing degrees of cluster membership.

In contrast to clustering, ordination seeks to reveal continuous, overlapping patterns of variation by arranging individuals in reduced low-dimensionality space. Distance data are represented such that the distances between individual plants in the reduced space resemble the raw distance values as closely as possible (see Cox and Cox, 1994). A number of ordination techniques have been utilised in hybridity studies, including multidimensional scaling (MDS), detrended correspondence analysis (DCA) and principal component analysis

(PCA) (e.g., Adams, 1982; Brochmann, 1987; Hawkins *et al.*, 1999). Ordination methods assume that groups cannot be identified *a priori*. Classical metric MDS (or principal coordinate analysis) operates on the absolute distances, whereas non-metric MDS considers only the rank order of the distances. DCA also reveals continuous variation patterns but OTUs and characters are plotted on the same axes, providing information on the relative contribution of individual characters to each axis. DCA operates on discrete characters and handles ordered multistate characters particularly well. Although rarely applied to studies of hybrids, Parnell and Waldren (1996) advocated the potential of DCA for such purposes over MDS and PCA, but in certain respects it is identical to MDS (the ordinations are based on equal character weighting and overall resemblance) and might be less robust than non-metric MDS (Faith, 1997). The outcome of classical metric MDS and PCA is equivalent when dissimilarities and Euclidean distances are identical (see Cox and Cox, 1994 pp. 34-35). However, PCA is not optimal for analysing hybridity data once groups are known (e.g., Pimentel, 1981; Adams, 1982; Brochmann, 1987).

Minimal spanning trees have occasionally been used in hybridity studies (e.g., Dancik and Barnes, 1975). Lines (or 'edges') connect the most similar points on a plot such that only a single path is possible and the tree has the minimal sum of the lengths of the edges. They can be useful for identifying distortion in ordination diagrams and for providing an additional perspective on taxonomic relationships (Sneath and Sokal, 1973 pp. 255-256).

Split decomposition is a recently developed tree-construction method for analysing distance or sequence data (Bandelt and Dress, 1992). The distances are transformed into weighted 'splits' and visualised by means of a 'splits graph', which is a tree or tree-like network (see Appendix 1 for a fuller synopsis of the method). It has the advantage of not forcing data into fully resolved dichotomous trees, allowing representation of reticulate relationships or alternative, conflicting hypotheses. Split decomposition has been used to aid identification of hybrids in *Fallopia* Adans. (Hollingsworth *et al.*, 1998) and *Lens* L. (Ahmad *et al.*, 1996; Ahmad *et al.*, 1997). Bandelt and Dress (1992) noted the potential application of split decomposition for detecting reticulate evolution and hybridisation events, but also noted the potential difficulty of distinguishing random and systematic error from hybridisation events.

The HYWIN computer program was conceived as an objective method for hypothesising which individuals might be of hybrid origin and for identifying the individuals most likely to be the parents from a set of morphological data (Estabrook *et al.*, 1996). It has been utilised,

in conjunction with canonical discriminant analysis, to aid identification of *Grindelia* and *Rhamnus* L. hybrids (Gil-ad and Reznicek, 1997; Tortosa *et al.*, 2000). The program considers each individual as a possible hybrid and examines the hypothesis that a particular pairing of the remaining individuals are possible parents, testing all possible combinations in turn. For each triplet a hybrid optimality score is calculated and weighted based on three criteria: hybrid intermediacy (wI); distance between the parental species (wP); and equidistance of the hybrid between the parents (wE). The triplets are then ranked based on the hybrid optimality scores, with the highest-ranked combinations representing the most plausible hypotheses.

3.4 Character descriptions

The morphological and anatomical characters found to be useful in the case studies are described below.

Growth form

A number of distinct growth forms occur among the New Zealand Gnaphalieae. The term 'mat' is used for plants that form flat carpets with a height difference between the plant centre and perimeter of less than 10% of the diameter. The shoot tips may be densely packed, e.g. *Raoulia grandiflora* (Plate 10 D, p. 218), or more loosely packed, as in *Anaphalioides bellidioides* (Plate 3 A, p. 140). Adventitious roots are produced at the nodes. In most species morphologically distinct prostrate and erect shoots are produced. 'Cushion' plants differ from mats in forming a convex mound and the difference in height between the edges and center is greater than 10% of the plant diameter. The shoot tips are tightly to densely packed. *Raoulia eximia* exemplifies this growth form (Plate 10 E). A shrub is defined as a woody, tap-rooted perennial lacking morphologically distinct prostrate and erect shoots, and usually lacking adventitious roots. This growth form is illustrated by *Ozothamnus leptophyllus* (Plate 2 F, p. 106). The term 'subshrub' is applied to plants that are similar to shrubs, but which have a more spreading habit, branches are only distinctly woody towards the base and adventitious roots may be produced from the stem bases on established plants. *Ewartia sinclairii* is an example (Plate 2 C).

Branching pattern

Most New Zealand Gnaphalieae produce two types of morphologically distinct shoots: non-flowering shoots, whose main function is spatial increment; and flowering shoots, which bear the capitula. The morphology and orientation of these shoot types varies. In the mat-forming species and stoloniferous *Euchiton* species, the non-flowering shoots are prostrate and have

longer internodes and smaller or scale-like leaves; the flowering shoots are erect, have larger leaves, and, at least prior to fruiting, have shorter internodes. The non-flowering shoots are produced from the lower leaf axils of flowering shoots and after growing a certain distance the tropism of the shoot tip changes to give rise to a new flowering shoot, which may flower during the following growing season. In some species, flowering shoots also develop from axillary buds on the non-flowering shoots. In *Anaphalioides bellidioides*, the flowering shoots are initially decumbent or prostrate and produce nodal adventitious roots; the apex only becomes truly orthotropic with development of the capitulum. In *Ozothamnus leptophyllus* both shoot types are erect; the non-flowering shoots have slightly larger leaves and longer internodes. In some species, such as *Anaphalioides bellidioides*, *Helichrysum filicaule* and *Leucogenes* species, the non-flowering shoots are produced at or below ground level from old wood. Thus the orientation of the shoots can be: prostrate (i.e., growing over the soil surface); decumbent (stems that are basally prostrate with an ascending shoot tip, e.g., the flowering shoots of *Anaphalioides bellidioides*); radial (the uppermost shoots are erect, the lowermost horizontal, with a continuum between the two, as in cushion-forming species); or erect (stems ascending or vertical in their entirety). The shoot tips are either densely packed (no discernible gaps around individual shoots, e.g. *Raoulia eximia*), loosely packed (plants are well branched but with distinct spaces around individual shoots, e.g., *Anaphalioides bellidioides* and the whipcord helichrysums), or sparse (the shoot tips are distantly spaced, e.g., *Helichrysum dimorphum*).

Rooting pattern

Most mat-forming species precociously produce adventitious roots from the nodes of prostrate stems. These roots are usually single, long, and sparsely branched or unbranched. Similar roots are produced towards the shoot tips in cushion-forming species and grow down through the dead leaves in the centre of the plant. Both rooting patterns are classified as 'nodal' in this thesis. In some species 'basal' adventitious roots are produced only from woody stems that touch the substrate but not precociously near the shoot tip, e.g., *Ewartia sinclairii* and *Helichrysum intermedium*. Other species are tap-rooted and lack adventitious roots (here termed a 'central' rooting pattern), as in *Ozothamnus leptophyllus*.

Juvenile phase

Some New Zealand Gnaphalieae possess morphologically distinct juvenile and adult growth forms. Cuttings taken from adults of such species often revert to a juvenile-like form and thus the presence of heteroblasty can be intimated without growing plants from seeds. Knowledge

of the juvenile form can be extremely important when identifying a putative hybrid in the indigenous Gnaphalieae.

The whipcord *Helichrysum* species have the most morphologically distinct juvenile form. The leaves of adult plants are tightly appressed to the stem and densely tomentose on the adaxial surface only, but juvenile plants have spreading leaves that are densely tomentose on both leaf surfaces. The transition from juvenile to adult is usually abrupt. In *H. intermedium* and *H. parvifolium* seedlings the juvenile form lasts for one year. In *H. dimorphum* the juvenile form resembles the broad leaf form of the adult. In some genera the juvenile phase is extremely brief. In *Euchiton limosus* seedlings, for example, the first leaves have long narrow petioles but from the eighth to tenth nodes they abruptly adopt the adult leaf form. In other genera (e.g., *Anaphalioides* and *Ewartia*), the leaves of juveniles and adults are not markedly different.

Internodes and leaf arrangement

The internodes are visibly distinct in some genera (e.g., *Anaphalioides*), but in many genera, such as *Helichrysum* and *Raoulia*, the internodes are often extremely short and the leaf bases overlap. The density of indumentum on the internodes can be dense, moderate, sparse or glabrous. The colour of the internode after removal of any indumentum is typically pale green but contains reddish pigmentation in some species. The angle between the adaxial leaf surface and the stem on primary shoots varies among species from less than 90° (i.e., leaves appressed to the stem or ascending), ± 90° (i.e., leaves plane or perpendicular to the stem), to greater than 90° (i.e., leaves descending).

Leaf lamina

The distal, photosynthetic portion of the leaf is defined as the lamina and the proximal, non-photosynthetic portion of the leaf is termed the petiole. However, the line of division between the lamina and petiole is indistinct in many New Zealand Gnaphalieae. A descriptive term that best described the shape of the whole leaf (i.e., lamina and petiole collectively) followed Stearn (1992).

In all New Zealand Gnaphalieae the lamina is simple and entire. The maximum lamina width was measured and a descriptive term following Stearn (1992) was used to describe the angle of the lamina margins relative to the blade. The lamina tapers evenly towards the petiole or narrows more abruptly (see Figure 4.12 p. 145 & Figure 5.1 p. 221). In some species hyaline

margins are present on the lower lamina and petiole. The point of maximum lamina width is defined as the distance from the lamina tip (excluding the mucro) along the midrib at which the lamina is broadest.

Descriptive terms following Stearn (1992) were used to describe the shape of the lamina tip. The angle of the lamina tip relative to the leaf axis is either plane with the axis (the angle between the leaf base and leaf tip is ± 180°) or downturned (the angle is greater than 180°).

Petiole

In most indigenous Gnaphalieae the petiole forms a sheath that envelops the stem to varying extents. In some species the petiole extends below the node in the form of two tiny 'wings' or ridges (Figure 3.1). The length of the petiole extensions below the node and their degree of sheathing around the stem varies among species. The length of the extensions below the node and the degree of sheathing was estimated as a percentage of the total length or circumference of the internode.

Mucro

The mucro is a hard, short protrusion at the lamina tip. It is an extension of the midrib and is typically pale green, but possesses a reddish pigmentation in some species. In many New

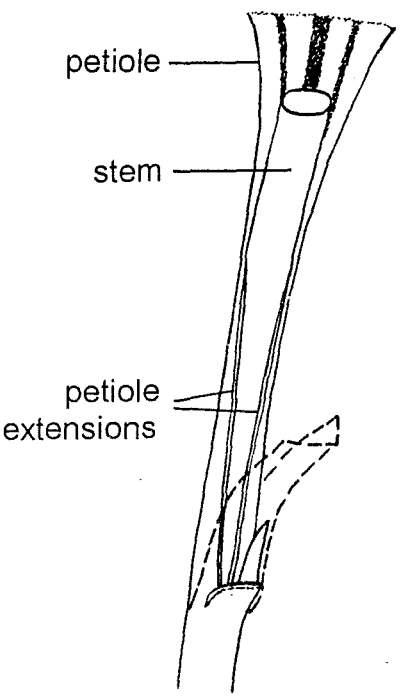


Figure 3.1. Leaf petiole extensions in *Anaphalioides bellidioides*.

Zealand Gnaphalieae it is very distinct and the length varies among species, but in some species it is absent. In some putative hybrids the mucro is vestigial or the leaf tip is attenuate and so the mucro is more difficult to measure. The mucro ends where the lamina tip broadens and photosynthetic tissue is clearly visible on either side of the midrib. In *Ewartia sinclairii* the mucro is strongly reflexed towards the adaxial leaf base (i.e., the angle formed between the mucro tip and the adaxial lamina surface is less than 45°). In most other mucronate species the mucro is erect or plane with the leaf axis (i.e., the angle between the mucro tip and the adaxial lamina surface is 90° or greater).

Leaf venation

Leaf venation in the New Zealand Gnaphalieae is of the brochidodromous camptodromous type, in which the secondary veins do not terminate at the margin, but are instead united in a series of arches (Hickey, 1979), thus producing a looped pattern (see Figure 4.12 p. 145 & Figure 5.1 p. 221). In *Raoulia* the overall venation pattern may be reticulate, semi-reticulate or striate (Solbrig, 1960). The number of primary veins entering the leaf is always one or three and is consistent for a given species. The higher-order venation pattern (i.e., secondary veins and above) is either predominantly parallel, or predominantly looped or reticulate. The ratio between the point of higher-order nerve branching from the leaf base and the total leaf length varies among species. In some species the nerves are visibly raised on the adaxial surface (e.g., whipcord *Helichrysum* species). The midrib is raised on the abaxial surface in many species, as are the lateral primary nerves in some species (e.g., *Ewartia sinclairii*).

Leaf trichomes

A range of glandular and non-glandular trichomes is recorded on the leaves in the Compositae (Ramayya, 1962; Theobald *et al.*, 1979). Following the terminology of Solereder (1908), two classes of trichome (clothing trichomes and glandular trichomes) are distinguished.

Clothing trichomes are present on at least one leaf surface and often form a conspicuous felt-like indumentum. They are uniseriate with one to three short basal cells (see Figure 5.3 p. 222) and a much longer, thread-like apical cell. The term 'clothing trichome' is used to avoid any confusion with the uniseriate multicellular trichomes found on *Raoulia grandiflora* leaves. The structure of clothing trichomes is variable in the Compositae (Theobald *et al.*, 1979) and two main forms occur in the New Zealand Gnaphalieae (Solbrig, 1960; Ward, 1993a). In the typical form (here termed 'type A'), the terminal cells are narrow (up to about 10 μm wide), whip-like and usually densely interwoven and appressed to the leaf surface. In

the form designated 'type B', the trichomes are broader (up to about 45 μm wide), thicker walled and rather rigid, and the terminal cells are shorter and usually not interwoven. The number of basal cells and their total length is variable among species. The junction between the terminal and basal cells is either swollen (i.e., broader than the basal cells) or the same width as the basal cells. The degree of appression of the terminal cells ranges from tightly appressed to loosely or not appressed. In some species the clothing trichomes conceal the mucro.

Simple biseriate trichomes occur in many genera of the Compositae (Metcalfe and Chalk, 1979). They appear to be present on the leaves of all New Zealand Gnaphalieae but are usually concealed by the clothing trichomes. In all but one species studied, these trichomes are consistently biseriate. In *Raoulia grandiflora*, however, all but two of over 100 trichomes observed were uniseriate. It is for this reason the term 'glandular trichome' is used, rather than 'biseriate trichome', even though a glandular function has not been demonstrated. However, structurally similar trichomes found in other Compositae are classified as glandular by other authors (e.g., Hummel and Staesche, 1962; Lundgren, 1972; Metcalfe and Chalk, 1979). Three distinct forms of glandular trichomes were identified among the species studied. In the most common form (designated 'type A'), the trichomes are biseriate, narrowest at the base or of uniform width throughout their length, and the terminal cells are oblong or slightly swollen (Figure 4.14 A–C p. 146). 'Type B' trichomes are biseriate, broadest at the base and narrowest at the apex, often curved and the terminal cells are smaller than the basal cells (Figure 4.14 D–E). In a third form ('type C'), observed only in *Raoulia grandiflora*, the trichomes are long, uniseriate and very thin walled. The terminal cell is oblong but not swollen. Thus the number of cell series, the width at the base, midpoint and apex, the total length of the trichome, and the length and width of the terminal cells is variable among indigenous Gnaphalieae.

In addition, the distribution and density of both clothing and glandular trichomes is often variable (from dense to absent) on both the laminar (i.e., photosynthetic) and petiolar (i.e., translucent) regions, and the adaxial and abaxial surfaces, of the leaf.

Leaf anatomy

The leaves of the New Zealand Gnaphalieae vary considerably in anatomy and thus offer a number of potentially important characters for hybridity studies. The following characters were selected from those described by Breitwieser (1993). The relative thickness of the cuticle and epidermis on the adaxial and abaxial lamina surfaces, the existence of distinct

palisade and spongy chlorenchyma layers, the presence of palisade chlorenchyma in the midrib, and the size and shape of the chlorenchyma cells vary among species. The guard cells and adjacent epidermal cells are raised above the epidermis in some species but level with the epidermis in other species. The presence and distribution of collenchyma and sclerenchyma in the midrib and lateral ribs are particularly variable among the New Zealand Gnaphalieae. In addition, idioblastic sclereids are present in the petiole on either side of the midrib in some *Raoulia* species (Solbrig, 1960).

Capitulum

In the Compositae numerous small florets are borne in a compact involucrate head called a capitulum. Ligulate florets are absent in the capitula of the Gnaphalieae. In some genera the bracts surrounding the capitulum have hygroscopic, radiate tips so that the capitulum superficially resembles a capitulum with ligulate florets (Plate 4 A p. 142). The capitula are borne singly on each shoot (i.e., solitary) in many indigenous Gnaphalieae, but in some species (e.g., *Ewartia sinclairii* and *Leucogenes* species), multiple capitula are borne in cymose aggregations (here termed inflorescences) on each flowering shoot. In *Leucogenes* the upper internodes are compressed and the capitula are subtended by large, felted bracts, forming an inflorescence that superficially resembles a radiate capitulum (Plate 12 A p. 220). The capitula are either sessile (e.g., whipcord *Helichrysum* species), pedunculate at anthesis (e.g., *Ewartia sinclairii*) or become pedunculate prior to fruiting (e.g., *Euchiton* species). In most pedunculate species the peduncle elongates with capitulum age.

Floret types

In all New Zealand Gnaphalieae so far investigated, with the possible exception of *Raoulia haastii*, flower development within the capitulum is centripetal, i.e. the peripheral florets mature first and the central florets last (Wilton, 1997). Many New Zealand Gnaphalieae are gynomonoecious: the peripheral florets within a capitulum are functionally and structurally female; the central florets are structurally hermaphrodite but in different species are functionally male or hermaphrodite. Wilton (1997) termed the two floret types 'filiform' and 'tubular' respectively on account of the differing corolla tube shape, but in this thesis 'female' and 'hermaphrodite' are used and refer only to floret *structure*, not the functioning of the sexes. The proportion of female to hermaphrodite florets within each capitulum varies considerably among the New Zealand Gnaphalieae and a few species, such as *Ozothamnus leptophyllus*, produce structurally hermaphrodite florets only.

Receptacle

The axis to which the florets and involucral bracts are attached is termed the receptacle, although because a capitulum is an inflorescence this is not homologous with a flower receptacle. All receptacle characters were described from capitula at anthesis. The curvature of the receptacle varies among species from plane to convex or conical. The surface of the receptacle varies in morphology, terms for which follow Small (1917). The point of floret attachment can be slightly raised and surrounded by low furrows (termed 'scrobiculate') (Plate 5 A p. 143). The point of floret attachment can be surrounded by low, narrow ridges and the depression circular ('areolate') to polygonal ('foveolate') (Plate 5 C & D). A ring of tiny scale-like structures can surround the base of each floret ('fimbrillate') (Plate 5 B).

Receptacular scales (or paleae) are morphologically similar to involucral bracts but are attached to the receptacle among the florets and each scale subtends a single floret. They are present in numerous genera in the Gnaphalieae (see Anderberg, 1991; Anderberg, 1994), but of the New Zealand Gnaphalieae studied in this thesis, they are present in *Ozothamnus leptophyllus* only.

Involucral bracts

In the Gnaphalieae the capitulum is surrounded by an involucre of bracts with usually papery tips. Drury (1970) distinguished three regions in each bract. The 'stereome' is the rigid, basal portion of the bract and is usually green at anthesis. Hyaline margins are present on the stereome of many species. The 'lamina' is the papery, terminal part of the bract. In the species studied in this thesis, the lamina is white or shades of yellow or brown. The 'lamina-stereome gap' is a hyaline region that may have additional pigmentation in reddish-purple or brown shades. Inner bracts (usually the longest and narrowest and with the longest lamina) and outer bracts (the lowermost on the receptacle and with usually a short lamina) are distinguished. In some species, such as *Anaphalioides* species and *Ewartia sinclairii*, the lamina is hygroscopic, opening during the day and closing at night or during high humidity. The shape of the lamina apex varies, for which a descriptive term following Stearn (1992) was used, and the presence and width of the hyaline margins on the stereome also vary among speces.

Corolla

The corolla forms a narrow tube with usually four lobes in female florets and five lobes in hermaphrodite florets. The corolla tube is narrower in structurally female florets than in structurally hermaphrodite florets (Figure 4.20 p. 150). The tube is broadest at the base of the

corolla lobes. The lower part of the corolla tube is usually white or pale green. The colour of the corolla lobes at anthesis differs among species, from pale green to white, yellow or crimson, and occasionally the corolla lobes develop reddish pigmentation after anthesis (e.g., *Anaphalioides bellidioides*). The upper corolla tube is reddish-purple at anthesis in some species (e.g., *Ewartia sinclairii*). The orientation of the corolla lobes at anthesis varies among species from erect to patent or recurved.

Ovary

The outer wall of the epidermal cells can be papillate (i.e., the distal end of the cell protrudes and covers the base of the adjacent cell), rounded or flat. The ovary is glabrous in some species, but 'twin hairs' (or duplex hairs) are present on the epidermis in other species (Plate 7 A–D p. 184). Twin hairs are a type of biseriate trichome with usually one (rarely more) small, thick-walled basal cell and two elongate terminal cells with extremely thick walls (Hess, 1938). Twin hairs comprising more than three cells are termed multicellular in this thesis. Shorter, bicellular twin hairs are also occasionally present. The shape of the tip of the terminal cells varies among species, for which a descriptive term following Stearn (1992) was used. The terminal cells are either coherent to the tip or free at the tip. The length and width of the ovary varies between species. The mature fruit is termed a 'cypsela' and contains a single seed.

Pappus hairs

The pappus hairs are generally interpreted as modified sepals and form a ring at the top of the ovary. In some species the pappus hairs are distinctly dimorphic on the same floret. The shape of the apical cells is clavate or acute and the number of apical cells varies among species (Figure 4.23 p. 152 & Figure 5.8 p. 225). The walls of the apical cells are uniformly thickened with obvious pits (termed 'uniform') in some species, but in other species the wall thickening is irregular (giving a sculptured appearance) or forms a distinct network pattern (both are termed 'reticulate'). In some species the apical cells protrude from the pappus axis. Distinct, cellular spines project from the base of the pappus axis. Spines whose length is at least equal to the width of the pappus axis have been termed 'cilia' (Drury, 1970). The density of the spines varies among species from dense to sparse. The angle between the pappus axis and the inner surface of the spine varies from ascending (± 45° or less), spreading (± 90°) or recurved (greater than 90°).

Other floral characters

Other floral characters recorded were: the colour of the pollen grains, the colour of the anthers; and the colour and length of the style arms.

Characters rejected or not investigated

Internode length was not measured owing to the difficulty of determining internode number from the shoot apex, which would ensure comparability. Because the line of division between the lamina and petiole is indistinct in many New Zealand Gnaphalieae, the lamina and petiole lengths were not measured separately. The morphology of the style arms, stamens and pollen were not investigated. The total number of involucral bracts per capitulum and pappus hairs per floret were not recorded. Continuous characters rejected in one or both case studies because the differences between species were too slight to allow discrimination of an intermediate state were: corolla lobe dimensions, corolla tube width and style arm length.

Chapter 4. Case study 1: *Anaphalioides bellidioides* (G.Forst.) Glenny × *Ewartia sinclairii* (Hook.f.) Cheeseman

4.1 Introduction

Anaphalioides bellidioides has a widespread distribution, extending from Urewera in the central North Island southwards to the subantarctic islands and occurring from lowland to alpine altitudes (Glenny, 1997). In contrast, *Ewartia sinclairii* is confined to the Awatere and inner Clarence drainage areas in Marlborough and grows at montane to subalpine altitudes (Allan, 1961). *A. bellidioides* grows in damp, but well-drained, situations in a range of habitats, including scrubby areas, grassland, streamside banks and stable scree margins, whereas *E. sinclairii* prefers rocky, partly shaded sites. The two species are not commonly sympatric (J. M. Ward, pers. comm.).

Anaphalioides bellidioides was described as a species of *Xeranthemum* by Forster (1786), but following redefinition of Linnaean genera it was subsequently transferred to *Helichrysum* by Willdenow (1800), *Gnaphalium* by Hooker (1853), then back to *Helichrysum* by Bentham (1873). Anderberg (1991) considered it belonged in the *Lawrencella* complex, but it has recently been placed in the genus *Anaphalioides* (Glenny, 1997). Mueller (1889) transferred a separate species, *Gnaphalium prostratum* (Hook.f.) Hook.f., to *Anaphalis*, but it is now considered synonymous with *A. bellidioides* (Glenny, 1997).

Ewartia sinclairii was described by Hooker (1864) as *Gnaphalium* (*Helichrysum*) *sinclairii*. Hooker considered it was closely allied to the Tasmanian *Raoulia catipes*, which was later transferred to *Ewartia* by Beauverd (1910). Kirk (1899) and Cheeseman (1906) included *E. sinclairii* in *Helichrysum*, although neither saw specimens and used Hooker's description. Cheeseman (1925) appears to have been influenced by Hooker's comments and transferred the species to *Ewartia*, commenting (p. 981), "the structure of the flower-heads of *E. sinclairii* corresponds with that of *E. catipes* in all essential points". *Ewartia sinclairii*, however, lacks Beauverd's (1910) defining generic character for *Ewartia*, namely subdioecy, and recent studies (Anderberg, 1991, but see Puttock, 1994; Breitwieser and Ward, 1993; Ward, 1993b; Glenny and Wagstaff, 1997; Breitwieser *et al.*, 1999) suggest it is misplaced in *Ewartia*. Anderberg (1991) erected the monotypic genus *Ewartiothamnus* to accommodate it, but Ward and Breitwieser (1998a) retained *E. sinclairii* in *Ewartia* pending resolution of its generic affinities.

Thus in the past both species have been placed in the genera *Gnaphalium* and *Helichrysum*, but no author has explicitly considered the two species to be closely related based on comparative morphology. Hooker (1864) placed both species in *Gnaphalium* and stated (p. 151), "those [species] with white radiating involucral scales form, I think, a most natural genus or group", but he also clearly stated his opinion that *E. sinclairii* is very closely allied to the Tasmanian *E. catipes*. Kirk (1899) and Cheeseman (1906) included the two species in *Helichrysum* but made no comment on their relationship. Cheeseman (1925) reiterated Cockayne's (1922) opinion that *H. fowerakeri* (at that time known only from a single plant) was a probable hybrid between *A. bellidioides* and *E. sinclairii*, but still transferred *E. sinclairii* to *Ewartia* while retaining *A. bellidioides* in *Helichrysum*.

Putative hybrids between *A. bellidioides* and *E. sinclairii* are recorded from five localities, all of which are within the geographical range of *E. sinclairii*. The first plant collected was described as a distinct species, *Helichrysum fowerakeri* (Cockayne, 1916), but in his description Cockayne noted its similarity to *A. bellidioides* and *E. sinclairii* and that it might be of hybrid origin. In later treatments *H. fowerakeri* is listed as a hybrid between *A. bellidioides* and *E. sinclairii* but no substantive data has been published (Cockayne, 1922; Cheeseman, 1925; Cockayne and Allan, 1934; Allan, 1961). Seven putative hybrids were collected on 21 November 1989 by Josephine Ward and John Lovis from the west branch of the Yeo Stream, Inland Kaikoura Range, the site of the present study. Other putative hybrids have been collected from the Awatere, Dee and Hodder river valleys (see section 4.3.7 p. 178).

The principal objective of this case study was to test the hybridity hypothesis using morphology and leaf anatomy data. The fertility of the putative hybrids and meiotic pairing were also evaluated, as abnormalities provide additional evidence for hybridity. Additional objectives were to obtain information on character expression in the putative hybrids and hybridisation barriers between the putative parents. The ability of seven multivariate analytic methods to identify the putative hybrids was also evaluated.

4.2 Materials and methods

4.2.1 The study site

I first visited the site of the original collection by Josephine Ward and John Lovis (the west branch of the Yeo Stream, Inland Kaikoura Range, Molesworth Ecological Region) on 28 December 1995. The putative hybrids were growing on the sparsely vegetated riverbank at

1100 m associated with low-growing grasses and other herbs. The following Gnaphalieae were growing on the bank: *Anaphalioides bellidioides*, *Ewartia sinclairii*, *Helichrysum coralloides*, *H. parvifolium* and *Ozothamnus leptophyllus*. No related species were growing on the riverbed in the vicinity. Capitula were present on plants of *A. bellidioides*, *E. sinclairii* and two putative hybrids, but none of the other species were flowering, although *H. coralloides* plants were in flower downstream.

Four putative *A. bellidioides* and *E. sinclairii* hybrids were growing on the riverbank; these were probably different plants to those collected in 1989 (J. D. Lovis, pers. comm.). All four were growing beside a drainage channel running down the rocky bank with the putative parental species growing close by. One putative hybrid (*W9* – see section 4.2.2 for an explanantion of hybrid code names) was a large plant, 40 cm in diameter, in full flower at the time, and was growing beside a group of *A. bellidioides* plants. A second putative hybrid (*W10*) was 30 cm in diameter but many of the shoots were dead. *W11* was a small seedling about 4 cm tall with three shoots growing in a shady nook to the right of the drainage channel. One of the shoots was collected as a cutting. Neither *W10* or *W11* was in flower. A fourth putative hybrid (*W12*) was completely dead except for a single, 4 cm long, lateral shoot bearing a single terminal capitulum. An additional putative hybrid (*W13*) was discovered by Grant Bawden downstream from the main hybrid site. The bank was south-facing, shady and more densely vegetated than the main site. *Ewartia sinclairii* and *H. parvifolium* plants were growing at the site, but no *A. bellidioides* individuals were located. The putative hybrid was not in flower.

4.2.2 Plant specimens available for study

Three groups among the putative hybrids are distinguished, based on the origin of the specimens studied (Table 4.1 p. 86). 'Field-grown' wild putative hybrids were described from specimens collected from plants *growing at the study site* (i.e., *W4*, *W5*, *W8*, *W9*, *W10* and *W12*). 'Seed-raised' putative hybrids were grown from seed collected from the study site (i.e., *S1*, *S2* and *S3*). The remaining wild putative hybrids ('field-collected') were growing at the study site but were described from *cultivated clones*. The seed-raised and field-collected plants are sometimes referred to as the 'cultivated' putative hybrids collectively. *S1* was raised from seeds from the sole capitulum of *W12*, and *S2* and *S3* were raised from *A. bellidioides* seeds collected from the study site. Two putative hybrids (*W1* and *W2*) from the original collection by Ward and Lovis were in cultivation at the University of Canterbury at the start of this thesis but neither bore a collection number, hence new vouchers were prepared and

Identification number in this thesis	Identification number or herbarium voucher number	Specimens from cultivated plants	FAA-preserved specimens from field-growing plants	Herbarium specimens from field-growing plants
Wild putative hybrids:				
W1	CANU 38498	+		
W2	CANU 38499	+		
W3	CANU 33077			+
W4	*J.M.Ward 89314*		+	
W5	CANU 33078		+	+
W6	CANU 32847			+
W7	CANU 32848			+
W8	*J.M.Ward 89318*		+	
W9	*R.J.McKenzie 138/1*	+	+	+
W10	*R.J.McKenzie 138/2*	+	+	+
W11	*R.J.McKenzie 138/3*	+		
W12	*R.J.McKenzie 138/4*		+	
W13	*R.J.McKenzie 138/5*	+		
Seed-raised putative hybrids:				
S1	*R.J.McKenzie 265*	+		
S2	*R.J.McKenzie 281/1*	+		
S3	*R.J.McKenzie 281/2*	+		

Table 4.1. Specimens of putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii* from the Yeo Stream available for study in this thesis.

new University of Canterbury Herbarium numbers allocated. It was not possible to propagate *W12* and preparation of a herbarium voucher was impossible, so only FAA-preserved material exists for this plant.

A small number of mature cypselas were obtained from the sole capitulum collected from *W12* prior to it being preserved in FAA. These were sown on 3 January 1996. Seven seeds germinated, but only one seedling (*S1*) was strong enough to reach maturity; the remaining seedlings were weak and died at the cotyledon or first-true-leaf stages. As mentioned above, when the seeds were collected *A. bellidioides*, *E. sinclairii* and the putative hybrid *W9* had also flowered, any of which may thus be the paternal parent of *S1*. Seeds were also collected from *A. bellidioides* plants growing at the main hybrid site and sown on 3 January 1996. Two putative hybrids were among the plants raised and are also included in the study.

Flowering specimens were collected from all sympatric gnaphalioid species growing at the site during the 1998-99 summer. Capitula were collected from plants of *A. bellidioides*, *E. sinclairii*, *W9* and *W10* on 21 December 1998. Shoots from the later-flowering species (*H. coralloides*, *H. parvifolium* and *O. leptophyllus*) were collected on 24 January 1999, by which time all capitula of the earlier-flowering species had matured and the cypselas dispersed. No change in the study site nor additional hybrids were observed on either date.

Herbarium specimens of eight putative hybrids between *A. bellidioides* and *E. sinclairii* collected from other localities were also examined to determine their identity and assess variation among the putative hybrids. FAA-preserved specimens from a cultivated clone were available for study for one of the putative hybrids (CHR 385817).

4.2.3 Sampling strategy and character selection

Shoots from up to nine plants of each species growing in the vicinity of the putative hybrids were collected at random. For some species the sample size was limited either by the species' rarity at the site (*Anaphalioides bellidioides*) or the rarity of plants in flower (*Helichrysum coralloides* and *H. parvifolium*). However, all species studied were morphologically well differentiated, the populations at the study site were not visibly variable, and no evidence for introgression between species was observed. Each individual plant studied is treated as an 'operational taxonomic unit' or OTU (see Sneath and Sokal, 1973 pp. 68-71).

To minimise the risk of misidentification of the putative hybrids, 89 characters (comprising vegetative and floral, and continuous and discrete) were recorded. In the New Zealand Gnaphalieae several cross-compatible species are often sympatric, so it was important to identify characters that discriminate *all* sympatric species in order to gain evidence for hybridity and to identify the most likely parental species. All characters utilised are defined in Chapter 3. Character selection was subjective in order to minimise time and resources spent studying uninformative characters. Preference was given to easily accessible characters allowing clear discrimination of the sympatric species and of possible intermediate character states, as these are the most informative for detecting hybridity.

Characters were described from specimens collected from the study site ('field-grown plants') and from cultivated plants grown under uniform conditions. The latter enabled description of vegetative characters at comparable stages of maturity and in plants of similar vigour, and to eliminate environment-induced variation. For the initial comparison between the putative

hybrids and all sympatric Gnaphalieae, vegetative characters were described from cultivated plants. Since plants of *H. coralloides* and *H. parvifolium* did not flower in cultivation within the time available, floral characters were described from field-collected capitula. For two putative hybrids, characters recorded from field-grown and cultivated clones were compared. For four putative hybrids only FAA-preserved shoots from field-collected specimens were available and for seven putative hybrids only cultivated specimens were available. Pigmentation characters were described from fresh material, but for other characters FAA-preserved specimens were used. All capitula were measured during anthesis but prior to fruiting. For species with multicapitulate inflorescences, the terminal, earliest maturing capitulum from different inflorescences was preferentially measured. The width of the capitulum was measured across its longitudinal midpoint. The length of the capitulum was measured from its base to the tips of the involucral bracts, which were closed in those species with hygroscopic laminae. Ovary characters were described from florets at anthesis. The length of pappus spines was measured along the inner side of the spine.

4.2.4 Cultivation of plants

All cultivated plants were grown under uniform conditions in an unheated glasshouse on the campus. A standard growing mix, comprising 2 parts peat: 1 part SC5 metal chip: 1 part river sand and containing 10 g/l 8-9 month Osmocote®, was used for all plants. The surface of the mix was covered with a layer of sieved SC5 gravel to help keep the mix cool and retain moisture. The pots were plunged in a bed of SC5 gravel equipped with an automatic watering system to keep the gravel damp.

Field-collected seed was germinated on a mix comprising equal parts peat and perlite and containing 1 g/l Osmocote®. The mix was moistened with a fungicide, covered with river sand and the seeds sown on the surface. The covered seed trays were placed in a cool, shady position in the glasshouse until the seeds germinated. Seed from 12 experimental crosses involving the putative parental species and putative hybrids was sown on 20 April 1998 on sterilised germination pads in petri dishes. The dishes were placed on a bench in the laboratory out of direct sunlight and germinated at ambient temperature.

4.2.5 Microscopy

Wild M3C and Olympus VS-IV stereo microscopes were used for general observation and measurement of morphological characters, and also for experimental crosses (for

methodology, see p. 268–270). Anatomical and micromorphological characters and meiotic pairing were observed with Leitz DIAPLAN and Olympus CH compound microscopes.

Cold-stage electron microscopy was used to examine the receptacle surface and trichomes on the leaf and ovary. Fresh specimens were partially embedded in Aquadag (a carbon-based lubricant) on an aluminium stub, frozen in liquid nitrogen and coated with gold using a Polaron E5000 splutter coater. Specimens were viewed using a Leica S440 scanning electron microscope.

4.2.6 Leaf clearing

Leaves were cleared in order to observe leaf-trichome structure and leaf venation. The leaves were fixed in FAA, rehydrated in an ethanol dilution series (70 %, 50 %, 30 % and 10 %, with a minimum of 30 min in each solution) and rinsed twice in distilled water (the duration varied from 30 min each to overnight). The leaves were placed in 8 N NaOH at room temperature for up to 3 d, depending on the species, then rinsed twice in distilled water. Semi-permanent mounts were produced by mounting the leaves in glycerol and sealing the cover-slips with nail varnish. For examination of leaf-trichome structure, the leaves were observed under an Olympus BW2 compound microscope equipped with phase-contrast optics.

To investigate leaf venation, removal of the indumentum from both leaf surfaces was essential in order to discern the finest veins; this was removed after fixation to avoid damaging the leaf. The leaves were cleared in 8 N NaOH as above, after which any remaining indumentum was easily removed. The leaves were stained in 1 % aqueous safranin for 15–30 seconds and, if required, destained in 50 % ethanol to remove excessive background staining. Semi-permanent mounts were produced as above and viewed with a Wild M3C stereo microscope with a bottom light source.

4.2.7 Histology

Leaf anatomy was investigated in cultivated plants only. Specimens were embedded in Technovit 7100 methacrylate resin (Kulzer). Leaves were selected from young, vigorous plants and fixed in FAA under vacuum for 24 h. The indumentum was removed to help ensure the specimens infiltrated and embedded well and a section of the lamina midway between the base and apex was excised. Specimens were dehydrated via an ethanol series (70 %, 85 % and 100 %), transferred to fresh infiltrating solution (0.1 g hardener 2 per 10 ml resin) and refrigerated at 4 °C for at least 4 weeks. Fresh infiltrating solution was prepared prior to

embedding, and 200 µl hardener II per 15 ml infiltrating solution was added immediately before use. Specimens were placed in the embedding solution and left overnight for the resin to cure. Gelatine capsules were filled with Technovit 3040 backing resin (6 ml powder: 3 ml liquid), the specimens were placed on the gelatine capsule and left overnight to polymerise. Sections of 3-5 µm thickness were cut on a Jung rotary microtome equipped with a glass knife made with a LKB 2078 Histo Knife maker. Sections were stained with 0.5 % aqueous azur II (Gurr) and methylene blue (Gurr) for 15 seconds, rinsed with water and dried, and a coverslip fixed with DePeX mounting medium (Gurr).

4.2.8 Cytology

Capitula were harvested between 7 and 10 a.m. and fixed in 3 parts acetic acid: 1 part absolute ethanol. The involucral bracts were teased apart before placement in the fixative to ensure good penetration of the fixative. The capitula were stored in the fixative at 4 °C until examined. Anthers were dissected out, placed in a small drop of acetocarmine on a microscope slide and gently crushed with a spatula needle. A coverslip was applied, the slide was heated over a flame (but not to boiling point) and placed between two filter paper sheets, and gentle pressure was applied to the coverslip. This process was repeated if necessary until the cells were sufficiently flat. To produce permanent mounts, the position and orientation of the coverslip was marked on the slide with a diamond pen. The slide was immersed upside down in 45 % acetic acid. After the coverslip had floated off, both the slide and coverslip were dehydrated in absolute ethanol. A drop of euparol was immediately placed on the slide and the coverslip applied, ensuring it was positioned correctly. The slide was then left for at least one week before examination. The frequency of lagging chromosomes at anaphase I and micronuclei at telophase I, and of micronuclei at telophase II, was scored for 100 microsporocytes.

4.2.9 Pollen stainability

The pollen stainability of the cultivated putative hybrids was evaluated with Alexander's differential stain. The performance of the fluorochromatic reaction (FCR) was also tested. Alexander's differential stain indicates the presence of cytoplasm in intact pollen grains, whereas FCR indicates membrane integrity and esterase activity in pollen grains (Dafni and Firmage, 2000) and is thus a better indicator of pollen quality. Only freshly presented pollen was used.

Alexander's differential stain

A stock solution was prepared as described by Alexander (1980) and stored in a dark bottle. Chemicals were added in the following order: 20 ml 95 % ethanol, 20 mg malachite green (CI 42000, BDH Chemicals), 50 ml distilled water, 40 ml glycerol, 100 mg acid fuchsin (CI 42685, Raymond A. Lamb), 5 g phenol and 4 ml lactic acid. To avoid overstaining, a working solution of 3 ml stock solution: 2 ml glycerol was prepared and kept for 3-4 weeks before replacement. For each plant pollen was collected from six florets, each from separate capitula, stained with Alexander's differential stain and viewed after approximately 15 minutes. The proportion of normal and abnormal pollen grains was scored for 200 pollen grains per floret. The results were pooled and the overall means calculated for each plant. Normal pollen grains were filled with dense, red-staining cytoplasm, whereas the cytoplasm was shrunken, poorly stained or absent in abnormal grains. Pollen from the nine cultivated putative hybrids, four *A. bellidioides* plants and five *E. sinclairii* plants was stained.

Fluorochromatic reaction

The method followed Heslop-Harrison *et al.* (1984). A 2 mg/ml stock solution of fluoroscein diacetate (Sigma) in acetone was prepared in a vial covered with aluminium foil and stored at 4 °C. Immediately prior to use, the stock solution was added dropwise to sucrose solutions of different concentrations until they were permanently cloudy. Slides were examined with an Olympus BW2 compound microscope equipped with epifluorescence.

4.2.10 Analyses of morphological data

Seven analytic methods were utilised: simple numerical techniques (character count and hybrid index); dimension-reduction techniques operating on either the original data (canonical discriminant analysis) or distance estimates derived from the original data (cluster analysis, multidimensional scaling and split decomposition); and the HYWIN computer program.

Since most continuous characters recorded from field-grown and cultivated clones of two putative hybrids differed significantly (see pp. 98–99), characters recorded from cultivated plants and field-grown plants were analysed separately for all analytic methods used. Cultivated plants of *H. coralloides*, *H. parvifolium* and *O. leptophyllus* did not flower in cultivation within the time available, so data from field-grown specimens were analysed for the initial comparison of the putative hybrids and *all* sympatric species. Data from cultivated plants were utilised for the comparison between the putative hybrids and the two most likely parental species.

To ensure equal weighting of characters for the calculation of dissimilarities and character indices, the means of continuous characters were range-standardised between 0 and 1 using the formula $(x - b) / (a - b)$, where a is the maximum mean, b is the minimum mean and x is the mean for a character for each OTU. Discrete characters were also coded between 0 and 1; a single intermediate state was coded as 0.5, while two intermediate states were coded as 0.33 and 0.67. For presence/absence characters, 0 represented absence and 1 represented presence.

Data matrices comprising OTUs (rows) by characters (columns) were constructed. The character types included in the data set differed with the method of analysis (see Appendix 3). Mixed data were used for calculation of dissimilarities, character counts, character indices and HYWIN analyses. Only continuous characters were analysed by canonical discriminant analysis. Ratios were excluded from all analyses except character counts and canonical discriminant analyses. Counts were treated as continuous characters, as the sample frequency distributions were more similar to those of measurements and ratios than of characters recorded in discrete classes. Some continuous characters, such as the number of apical cells of the pappus hairs, were recorded as discrete variables.

Comparison of continuous characters from cultivated and field-grown clones of *W9* and *W10*

To evaluate the comparability of continuous characters recorded from field-grown and cultivated clones of the same plant, 15 continuous floral characters (consisting of ten measurements for each) were compared for *W9* and *W10*. Normal probability plots were produced with the STATISTIX 7.0 computer program (Analytical Software, 2000). Logarithmic or square-root transformation of non-normal characters and Student's *t* tests (with a 0.05 level of significance) were performed with the S-PLUS 4.5 computer program (MathSoft, 1997).

Character count

The procedure was based on the methods of Bateman & Farrington (1987) and Wilson (1992), but both continuous and discrete characters were included. Characters clearly discriminating the putative parental species were selected by the following criteria: continuous characters in which the standard deviation intervals (the mean ± the standard deviation) or median and first and third quartiles of the two species did not overlap; and discrete characters for which the parental species possessed distinct classes or states. For each continuous character, the overall mean, standard deviation, median and first and third quartiles were calculated for each species. Some continuous characters were not normally distributed, as indicated by normal

probability plots, and transformation did not improve normality, so two methods of determining the parental limits for continuous characters were used: standard deviation intervals centred on the mean; and first and third quartile intervals centred on the median. The putative hybrids were classified as: 'intermediate' if the character mean for the putative hybrid fell between the standard deviation or quartile intervals of the putative parental species; 'parental' if the mean fell within the standard deviation or quartile interval of either putative parent (termed 'equivocal' by Bateman and Farrington (1987) and Hawkins *et al.* (1999)); or 'extreme' if the mean fell beyond the uppermost or lowermost standard deviation or quartile limits of the putative parents. Discrete characters were classified as: 'intermediate' between the putative parent's states; 'parental' if they were identical to one of the putative parents; 'extreme' if they exceeded the range of variation of the putative parents; or 'novel' if the character state was unequivocally unique. If a putative hybrid possessed a novel character state, that character was excluded from the counts for other putative hybrids, as the character state was identical to *both* putative parents.

Character index

Calculation of character indices was based on the method of Brochmann (1987). Separate indices were derived from continuous, discrete and mixed characters. For continuous characters, the mean of up to ten measurements was used. Where necessary, characters were recoded so that *A. bellidioides* received the maximum value for each character and *E. sinclairii* the minimum value, but occasionally putative hybrids possessed the most extreme value. Continuous characters in which values were highest in *E. sinclairii* were recoded using the formula, $(a \times b) / x$, where a is the maximum mean, b is the minimum mean and x is the character mean for an OTU. Two characters unique to *S1* (the presence of multicellular twin hairs on the ovary of female and hermpharodite florets) were coded as 1 for *S1* and 0 for all other OTUs. All characters were range-standardised between 0 and 1 to ensure equal weighting. For each OTU the mean value of the transformed characters was calculated to obtain the character index value (or C-value). A histogram of C-value frequencies is the usual method of presenting the results, but owing to the small number of putative hybrids studied in this thesis, a histogram of individual C-values is presented. The frequency distribution of the character values for each individual was also examined; continuous characters were first recoded into discrete classes of interval 0.1 by rounding values to one decimal place. Data for three characters (receptacle diameter, female floret number and hermaphrodite floret number) were unavailable for one plant of *E. sinclairii*, but in all other instances characters containing missing data were excluded.

Canonical discriminant analysis

Multiple discriminant analysis was performed with the S-PLUS 2000 computer program (MathSoft, 1999) using a canonical homoscedastic model. A maximum of twenty continuous characters were included in the data set (see Appendix 3) and for each character the mean of up to ten measurements was used for each OTU. Ratios were included along with the numerator and denominator. Character distributions were assessed with normal probability plots and box plots. Characters with non-normal distributions or outliers were log or square-root transformed (see Appendix 3). A constant of 1 was added to characters containing values less than 1 prior to log transformation. Because *Ozothamnus leptophyllus* lacks female florets and discriminant analyses do not accept missing data, all female-floret characters (except the number of female florets per capitulum) were excluded from analyses containing *O. leptophyllus*. Discriminant analyses are very sensitive to outliers (Huberty, 1994 p. 64), so data sets were reanalysed with characters containing outliers excluded. Equality of group-covariance matrices is assumed (McLachlan, 1992 p. 88), so Box's M and adjusted M tests for covariance homogeneity were performed. Normality is required for the summary statistics and group-membership predictions calculated in S-PLUS, so normal probability plots and box plots were produced and non-normal characters were transformed (see Appendix 3). To test for equality of the group means, the Hotelling's T^2 test, Hotelling-Lawley trace, Pillai trace, Roy's greatest root and Wilk's lambda were calculated.

Discriminant functions were estimated from OTUs of all sympatric species and all available characters, then reestimated from a reduced data set comprising the three most likely parental species (the minimum number of groups allowable with the number of characters included). Plug-in classification, cross-validation and estimation of error rates based on posterior probabilities were performed in S-PLUS 2000 to estimate the misclassification rate and the discriminatory power of the discriminant function. Two-dimensional scatter plots of the canonical variates were produced to provide a visual representation of relationships. The contribution of each character to each canonical variate was determined by calculating Pearson's product-moment correlation coefficients between the original data and the canonical variates. Vector plots of the correlation coefficients were produced and for each canonical variate the characters were ranked by the absolute coefficient value. To predict group membership for each putative hybrid, plug-in classification was performed.

Calculation of dissimilarities

A matrix of dissimilarities was generated with the S-PLUS 4.5 computer program (MathSoft, 1997) using Gower's (1971) general coefficient of similarity and the Phenetic Library developed by Dr Aaron Wilton, Landcare Research New Zealand Ltd. For each continuous character, the mean of up to ten measurements was entered in the data matrix for each OTU. Where possible, the states for discrete characters were coded in a logical order. Continuous and ordered discrete characters were coded as character type 1 and unordered discrete characters as character type 2. No binary characters were included in the data sets, but characters with missing data were included.

Cluster analysis

Agglomerative and divisive hierarchical clustering, fuzzy partitioning and partitioning around medoids (PAM) were performed on the dissimilarities with the S-PLUS 4.5 computer program (MathSoft, 1997) using the hclust, diana, fanny and pam functions and the Phenetic Library developed by Dr Aaron Wilton. Complete, group-average, single and weighted-average linkage methods were compared for agglomerative clustering. The Spearman's rank and Pearson's product-moment correlation coefficients were calculated (using the cor.test function) as measures of the goodness of fit between the linkage levels and original dissimilarities. The divisive coefficient, which measures the clustering structure of the data set, was calculated in the divisive analysis. Dunn's partition coefficient was calculated as a measure of the 'crispness' of the clusters generated by fuzzy analysis. The silhouette coefficient (or overall average silhouette width) was calculated for each PAM analysis. For each coefficient, decreasing values indicate increasing distortion of the original dissimilarities. The putative hybrids were compared with the putative parental species only. For agglomerative methods, OTU randomisation was performed to assess whether the OTU order influenced phenogram structure and jackknife analysis of both OTUs and characters was performed as a measure of support for each cluster. Characters with missing data had to be excluded from the data set for the character-based jackknife analyses.

HYWIN

Three data sets, containing 22 continuous characters, 43 discrete characters and all (mixed) characters respectively, were analysed with the HYWIN computer program (Estabrook *et al.*, 1996). The program allows missing data so female-floret characters were included. To avoid character weighting, five characters (lamina width, hermphrodite floret number per capitulum, female:hermaphrodite floret ratio, female floret ovary width and hermaphrodite-floret ovary

width) were excluded from the data set. The characters leaf length:lamina width ratio, female floret number per capitulum, total floret number per capitulum, female-floret ovary length:width ratio and hermaphrodite-floret ovary length:width ratio (which canonical discriminant analysis indicated were better discriminators) were retained. Non-ordered multistate characters were excluded, since HYWIN is designed to test for intermediacy rather than similarity. The maximum number of characters was included, but accuracy is expected to improve proportionally less with increasing number of characters (Estabrook *et al.*, 1996). The program range-standardises the data and missing data are allowed. The intermediacy, equality and parental distance weightings were adjusted (from 0.1 to 1 for each criterion) to test whether HYWIN was informative for identifying groups among the putative hybrids. The 0.95 probability of all OTUs being ranked as hybrids was used to limit the number of hypotheses considered.

Metric multidimensional scaling

Classical metric multidimensional scaling (MDS) of the dissimilarities was performed with the S-PLUS 4.5 computer program (MathSoft, 1997) using the cmdscale function and the Phenetic Library developed by Dr Aaron Wilton. For each principal-coordinate axis, the proportion of the total variation and the proportion of the total sum of squared distances between points were calculated as measures of stress and to indicate the dimensionality required (Mardia *et al.*, 1979; Cox and Cox, 1994). Increasing sum of squared distance stress values indicate a decreasing goodness of fit between the dissimilarity matrix and the ordination. Three dimensions were selected for all analyses. Scatter plots of the principal coordinates for each OTU were produced and a minimal spanning tree was constructed on the scatter plots using the mstree function in S-PLUS 4.5.

Split decomposition

Split decomposition and the construction of splits graphs from the dissimilarities were performed with the SPLITSTREE 2.2 computer program (Huson, 1998). The refine option, with averages calculated over the maximum number of quartets (see Huson, 1998), was selected for all analyses. Species were sequentially excluded from the data set and dissimilarities recalculated to assess the impact on the structure of the splits graphs and placement of the putative hybrids in relation to the putative parental and non-parental species. An offset (e.g., 0.01) was added to the dissimilarities in all analyses to satisfy triangle inequalities. A Buneman tree, which comprises compatible splits only, was constructed in each analysis.

4.3 Results

4.3.1 Pollen stainability

Abnormal pollen grains were common for all of the cultivated putative hybrids, as indicated by Alexander's differential stain (Table 4.2). The proportion of normal pollen grains among the field-collected putative hybrids ranged from 43.7 % in *W11* to 60.8 % in *W10*. Of the seed-raised putative hybrids, nearly 70 % of the pollen grains were normal in *S1* and *S2*, but the frequency of abnormal grains in *S3* was similar to that of the field-collected putative hybrids. Normal pollen grains were filled with abundant red-staining cytoplasm and were 20–25 μm in diameter. Abnormal pollen grains were 10–20 μm in diameter. In some abnormal grains the cytoplasm stained strongly but was visibly shrunken from the pollen wall, while the smallest grains contained little or no stainable cytoplasm. Only 1.8 % of the pollen grains were abnormal in *A. bellidioides* and 0.7 % abnormal in *E. sinclairii*. The staining and size of normal and abnormal pollen grains in both species was identical to that of the putative hybrids. With the fluorochromatic reaction, the highest percentage of fluorescing grains recorded for the putative hybrid *W11* was 35 % after 2 h in a 2.5 M sucrose solution (see Appendix 4).

Putative hybrid	Normal pollen grains (%)
W1	46.9 ± 7.4
W2	47.7 ± 8.5
W9	49.9 ± 8.0
W10	60.8 ± 4.9
W11	43.7 ± 4.9
W13	58.9 ± 3.9
S1	68.8 ± 4.6
S2	68.7 ± 3.9
S3	54.5 ± 7.4

Table 4.2. Percentage of normal pollen grains in the cultivated putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, as indicated by Alexander's differential stain. The mean ± s. d. of 1200 pollen grains is presented for each putative hybrid.

4.3.2 Meiotic pairing in microsporocytes of *W9*

The florets of *W9* were 1.1–1.3 mm long when the microsporocytes underwent the meiotic division. Among these florets the stage of meiosis varied from interphase I to telophase II, sometimes among anthers of the same floret. Microsporocytes at diakinesis were rarely observed and in most at this stage the chromosome pairs were either insufficiently condensed or separated to permit unequivocal counts. One microsporocyte observed at diakinesis contained more than 14 (possibly 17) chromosomal entities (Plate 1 A p. 104). At metaphase I as many as five chromosomal bodies were irregularly positioned away from the equator of the spindle in many microsporocytes (Plate 1 B–E). Other chromosome pairs were closely aligned across the equator at metaphase I and unequivocal counts of chromosome pairings were not possible. Chromosome bridges were observed during anaphase I in three microsporocytes. Lagging chromosomes at anaphase I and micronuclei at telophase I (Plate 1 F) were observed in 20 % of the microsporocytes. The frequency of microsporocytes with micronuclei in telophase II was marginally higher (24 %). Eighteen per cent of the microsporocytes contained a single micronucleus, but up to four micronuclei were observed in a microsporocyte.

4.3.3 Morphology of all sympatric species and the field-grown putative hybrids

4.3.3.1 Comparison of continuous floral characters from cultivated and field-collected clones of *W9* and *W10*

Normal probability plots indicated that data for five characters (number of capitula per inflorescence, receptacle height, female floret pappus length, involucral bract length and involucral bract lamina length) were not normally distributed for at least one clone, but logarithmic transformation had little impact on the distribution. Therefore Student's *t* tests were not performed on these characters and two characters (number of capitula per inflorescence and involucral bract length) were excluded from the *t* tests.

For most characters, the means for the cultivated and field-collected clones were significantly different at a 0.05 level of significance (Table 4.3 p. 105). Three characters – receptacle diameter, female-floret number per capitulum and hermaphrodite-floret pappus length – did not differ significantly between cultivated and field-collected clones of *W9*. Five characters – number of hermaphrodite florets per capitulum, total floret number per capitulum, female-floret corolla tube length, female-floret pappus length and hermaphrodite-floret pappus length – were not significantly different between cultivated and field-collected clones of *W10*.

Hermaphrodite-floret pappus length was the only character that did not differ significantly between cultivated and field-collected clones of both plants.

4.3.3.2 Morphology of the putative hybrids and all sympatric Gnaphalieae

Morphological characters discriminating all sympatric Gnaphalieae and the putative hybrids are summarised in Table 4.4, Table 4.5 and Table 4.6 (following pp. 107–109).

Vegetative characters

The putative hybrids had a semi-prostrate to decumbent growth habit and produced adventitious root primordia near the shoot tips. *A. bellidioides* was the only sympatric species with a prostrate and precociously adventitious-rooting growth habit (Plate 2 p. 106). The other species were tap-rooted, erect shrubs (*H. coralloides, H. parvifolium* and *O. leptophyllus*) or decumbent to erect subshrubs that only produced adventitious roots at the base of mature shoots (*E. sinclairii*). The two *Helichrysum* species had a morphologically distinct juvenile phase, as determined from cultivating a seedling of *H. coralloides* collected from the Yeo valley and seedlings of *H. parvifolium* raised from seed collected from Mt McCabe, Marlborough by Aaron Wilton. The leaves of the juvenile were spreading with dense indumentum on both surfaces, whereas in the adult stage the leaves are tightly appressed to the stem and dense indumentum is present on the adaxial surface only. Most cuttings taken from adult plants of either *Helichrysum* species briefly reverted to the juvenile form. Seedlings of *A. bellidioides* (grown from seed collected from the study site) and *E. sinclairii* (grown from seed collected downstream from the study site and from the Hodder River) lacked a morphologically distinct juvenile phase. Cuttings of the field-collected putative hybrids, *A. bellidioides, E. sinclairii* and *O. leptophyllus* did not revert to such a phase.

In the *Helichrysum* species the leaves of the adult are appressed to the stem, the leaf tips are cucullate and the lamina margins are slightly involute. The leaves of *A. bellidioides, E. sinclairii, O. leptophyllus* and the putative hybrids are spreading and never appressed to the stem, and the leaf tip and lamina margins are plane. The lamina was obovate in the putative hybrids, *A. bellidioides, E. sinclairii* and *O. leptophyllus.* In *A. bellidioides* and some putative hybrids the lamina was distinctly narrowed above the petiole, but the degree of narrowing varied among the putative hybrids. In the other species the lamina tapered gradually towards the petiole. Extensions of the leaf petiole enclosed over 50 % of the stem in the putative hybrids, a characteristic shared only with *A. bellidioides.* The leaves of the putative hybrids also had a well-developed mucro, which in some putative hybrids was upturned. Only *A.*

bellidioides has a well-developed mucro and in the other species the mucro is considerably shorter or absent. The mucro of *E. sinclairii* was unique in being recurved. In some putative hybrids the mucro length slightly exceeded that of *A. bellidioides*. The leaf nerves were raised on the adaxial surface of the leaf in the *Helichrysum* species, but there was no evidence for this in the putative hybrids. The midrib, and in some plants the lateral nerves, were raised on the abaxial leaf surface in the putative hybrids, features in common with *E. sinclairii*. In *A. bellidioides* and *O. leptophyllus* only the midrib is raised. Neither the midrib nor the lateral nerves are evident on the abaxial leaf surface in the *Helichrysum* species.

The adult leaves of *H. coralloides* and *H. parvifolium* had dense indumentum on the adaxial surface but only sparse clothing trichomes on the abaxial surface. The leaves of *A. bellidioides* and *O. leptophyllus* had sparse indumentum on the adaxial surface and dense indumentum on the abaxial surface. Dense indumentum covered both leaf surfaces in *E. sinclairii*. In most putative hybrids the density of the indumentum was moderate on the adaxial surface and dense on the abaxial surface, but the leaves of *W3* had only sparse tomentum on the adaxial surface. Type B clothing trichomes were present at the leaf tip on the adaxial surface of the leaf in *H. coralloides* only. Type B glandular trichomes were present on the margins and adaxial surface of the leaf of most putative hybrids, a character shared only by *A. bellidioides*, in which they were larger and more frequent.

Leaf dimensions were compared separately for cultivated plants and for preserved field-grown specimens (see Table 4.5 & Table 4.6 following p. 107 & p. 108). Only *E. sinclairii* had longer leaves than the cultivated putative hybrids. Lamina width was similar in the cultivated putative hybrids, *A. bellidioides* and *E. sinclairii*. The leaves of *H. coralloides*, *H. parvifolium* and *O. leptophyllus* were shorter and narrower than those of the cultivated putative hybrids. The leaf length: width ratio was less informative for cultivated plants, owing to considerable overlap among the sympatric species. Leaf length and lamina width in the field-grown putative hybrids were similar to those of *A. bellidioides*, *H. coralloides* and *O. leptophyllus*. The leaves of *E. sinclairii* were longer and had a higher length: width ratio than those of the putative hybrids. The leaves of *H. parvifolium* were considerably shorter and narrower than those of the putative hybrids and the other species. The leaves of *W12* were notably smaller than the other field-grown putative hybrids. The length: width ratio in the putative hybrids was similar to *A. bellidioides* and *O. leptophyllus*; the ratio was higher in *E. sinclairii* and lower in the *Helichrysum* species. The point of maximum leaf width was always in the basal

half of the leaf in the *Helichrysum* species, but in all other species and the putative hybrids the point of maximum width: length ratio was similar.

Floral characters

The putative hybrids produced erect, morphologically distinct flowering shoots with narrow, acute, bract-like leaves. The number of capitula per shoot ranged from consistently one (in *W3*, *S2* and *S3*) to eight (in *S1*) and, where multiple shoots were available for study, varied on the same plant for individuals with multicapitulate inflorescences. *A. bellidioides* was the only sympatric species possessing erect, morphologically distinct flowering shoots with bracteate leaves, but the capitula are always solitary. *E. sinclairii* and *O. leptophyllus* bear multicapitulate inflorescences, whereas the *Helichrysum* species produce solitary capitula.

The capitulum length was similar among the putative hybrids. The capitula of *A. bellidioides* were longer and broader, and those of *E. sinclairii* and *O. leptophyllus* were shorter and narrower. The capitula of *H. coralloides* were longer but of a similar width to those of the putative hybrids. In *H. parvifolium* the capitula were narrower but of a similar length to those of the putative hybrids.

The receptacle was conical in *A. bellidioides* and *W3*, subconical in the other putative hybrids and *H. coralloides*, convex in *H. parvifolium* and *O. leptophyllus*, and flat or slightly convex in *E. sinclairii*. The receptacle was longer and broader in *A. bellidioides*, of similar dimensions in *H. coralloides*, and usually shorter and narrower in the other species. The receptacle was alveolate to foveolate in the putative hybrids, scrobiculate in *A. bellidioides* and fimbrillate in the other sympatric species. Receptacle scales were present in the capitula of *O. leptophyllus* only.

The inner involucral bracts of the putative hybrids had a white, hygroscopic lamina, a character shared with *A. bellidioides* and *E. sinclairii*. The bract lamina is also white in *O. leptophyllus* but is not distinctly hygroscopic. The shape of the lamina tip was obtuse to rounded in the putative hybrids, acute to obtuse in *A. bellidioides* and the *Helichrysum* species, and rounded in *E. sinclairii* and *O. leptophyllus*. The inner involucral bracts of the putative hybrids were intermediate in length between both putative parental species and similar in length to those of *H. coralloides*. The lamina was also intermediate in length between the putative parental species, but lamina width was similar in all species and the putative hybrids.

The number of female and hermaphrodite florets, and the total number of florets, per capitulum were highest in *A. bellidioides*. The number of hermaphrodite florets and total floret number per capitulum in *H. coralloides* were often higher than in the putative hybrids, but the number of female florets per capitulum was lower. The capitula of *E. sinclairii*, *H. parvifolium* and *O. leptophyllus* contained fewer female and hermaphrodite florets. The female: hermaphrodite floret ratio was similar among the putative hybrids; the ratio was higher in *A. bellidioides* but lower in all other sympatric species.

At anthesis the corolla lobes and style arms were greenish-white in the putative hybrids, green in *A. bellidioides*, white in *E. sinclairii* and *O. leptophyllus*, and yellow in the *Helichrysum* species. In *W1*, *W10*, *W12* and *S3* the corolla lobes became flushed crimson with age, a characteristic also present in some *A. bellidioides* plants. Crimson pigmentation was present in the upper corolla tube in *W3* and *E. sinclairii*. The corolla lobes were at least occasionally patent or recurved in all putative hybrids except *S1*. In *A. bellidioides* the corolla lobes were erect, but in all other sympatric species the corolla lobes were usually recurved. The corolla tube length in both floret types was similar in the putative hybrids, *A. bellidioides*, *E. sinclairii* and *O. leptophyllus*. The corolla tubes were longer in *H. coralloides* and *H. parvifolium*.

Crimson pigmentation was present in the anthers of most putative hybrids. The anthers of *E. sinclairii* were dark crimson, but in the other sympatric species the anthers were translucent. The colour of the pollen ranged from white in *E. sinclairii* and *W13*; pale yellow in *O. leptophyllus*, *W1*, *W2*, *W9*, *W10* and *W11*; and yellow in *A. bellidioides*, the *Helichrysum* species and the seed-raised putative hybrids (*S1*, *S2* and *S3*).

The pappus hairs of *W5*, *W10* and *W12* were similar in length to those of *A. bellidioides* and *H. coralloides*, whereas the other putative hybrids were similar to *H. parvifolium* and *O. leptophyllus*. *E. sinclairii* had the shortest pappus hairs for both floret types. The number of apical cells ranged from 1–2 in both floret types in *A. bellidioides* to 3–6 (in the female florets) and 5-8 (in the hermaphrodite florets) of *E. sinclairii*. The putative hybrids, *O. leptophyllus* and the *Helichrysum* species were intermediate between these extremes for both floret types. The shape of the apical cells were acute in *H. coralloides* and *H. parvifolium*, but clavate in the other species and all putative hybrids. The apical cells had distinctive reticulate wall thickening in *E. sinclairii* and *O. leptophyllus*, but were uniformly thickened in *A.*

bellidioides, the *Helichrysum* species and *W3*. In the other putative hybrids the apical cells had irregularly thickened walls. The pappus hairs of the female florets in *E. sinclairii* plants were unique in being distinctly dimorphic; the apical cells protruded in some hairs but not in others. In hermaphrodite florets the pappus-hair apical cells protruded in *E. sinclairii* and *O. leptophyllus*, but not in *A. bellidioides*, *H. coralloides* and *H. parvifolium*. The apical cells protruded in both floret types in *W1*, *W2*, *W6*, *W10* and *W13*, only in the hermaphrodite florets in *W4*, *W5*, *W7*, *W8*, *W9* and *W12*, and were not protruding in both floret types in *W3*, *S1*, *S2* and *S3*.

The ovary length in the female florets of most putative hybrids was similar to *A. bellidioides*, but in *W12* it was similar to *E. sinclairii*. The ovary length in the hermaphrodite florets of most putative hybrids was similar to that of *A. bellidioides*, *E. sinclairii* and *O. leptophyllus*, but in *W12* the ovaries were of similar length to those of the *Helichrysum* species. Ovary width in both the female and hermaphrodite florets was either similar to *A. bellidioides* (*W4*, *W5* and *W8*) or intermediate between *A. bellidioides* and the other sympatric species (*W9*, *W10* and *W12*). The ovary length:width ratio of both the female and hermaphrodite florets exhibited much greater variation among the putative hybrids and was less informative with regard to relationships.

The ovary of both the female and hermaphrodite florets was glabrous in *W3*, *W6*, *W7*, *W9*, *W10*, *W11* and *S3*, a feature shared with *A. bellidioides* and two individuals of *E. sinclairii*. Occasional twin hairs were observed on the ovary of female florets in *W1*, *W2*, *W5*, *W12*, *W13*, *S1* and *S2*, a feature shared with two plants of *E. sinclairii*. *S1* had occasional multicellular twin hairs on the ovary of hermaphrodite florets and a single such trichome was observed on the ovary of a female floret. Dense twin hairs were present on the ovary of both floret types in *H. coralloides* and *H. parvifolium*. Twin hairs were present on the ovary in *O. leptophyllus*, but, as mentioned above, this species lacks female florets. The twin hairs of the putative hybrids had clavate apical cells, as in *E. sinclairii*, *H. coralloides* and *O. leptophyllus*, whereas the apical cells were acute in *H. parvifolium*.

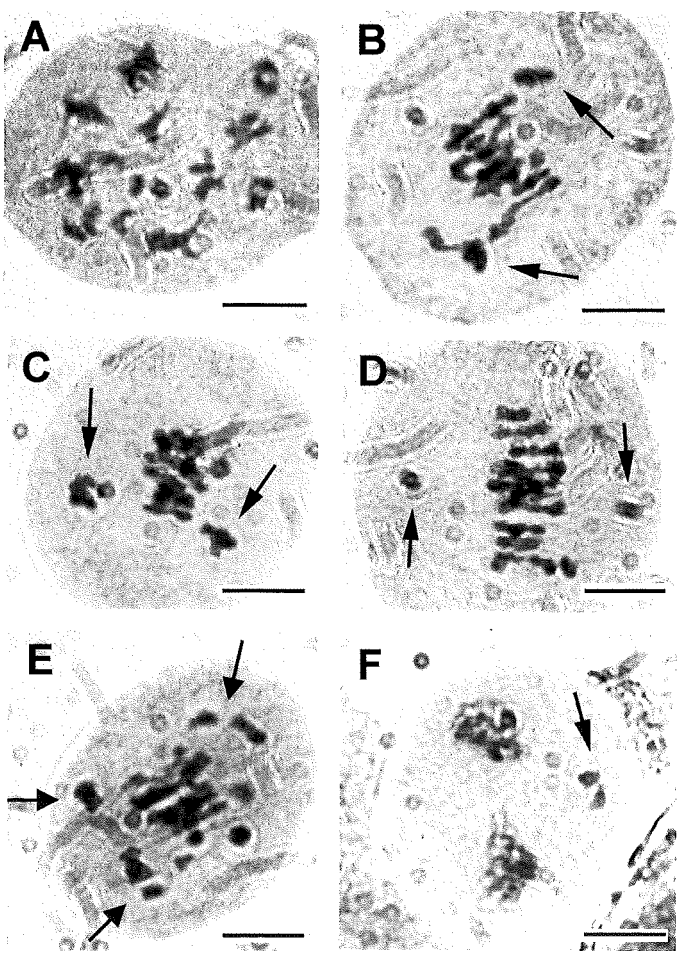
Plate 1. Meiosis in microsporocytes of the putative hybrid *W9*.

A, Diakinesis, microsporocyte with more than 14 chromosomal entities.

B – **E**, Metaphase I, showing irregular placement of some chromosomes away from the equator of the spindle (indicated by arrows).

F, Telophase II, microsporocyte with two isolated chromosomal entities (indicated by arrow).

Scale = 10 μm.


A
B
C
D
E
F

Character	*W9*					*W10*				
	Cultivated		Field-collected			Cultivated		Field-collected		
	Range	Mean ± s.d.	Range	Mean ± s.d.	P	Range	Mean ± s.d.	Range	Mean ± s.d.	P
capitulum length (mm)	6.3-6.8	6.56 ± 0.13	5.8-7	6.12 ± 0.22	***	6.9-7.5	7.11 ± 0.17	6.1-6.4	6.33 ± 0.20	***
capitulum width at midpoint (mm)	3-3.6	3.36 ± 0.18	3.2-4.6	3.74 ± 0.5	*	3.3-3.8	3.52 ± 0.16	4-4.5	4.21 ± 0.20	***
receptacle height (mm)	1-1.2	1.12 ± 0.08	0.65-0.88	0.74 ± 0.07	NT	1.08-1.25	1.19 ± 0.06	0.85-1.08	0.94 ± 0.09	***
receptacle diameter (mm)	1.6-2	1.74 ± 0.13	1.4-1.9	1.63 ± 0.14	*ns*	1.75-1.88	1.81 ± 0.05	1.6-1.75	1.63 ± 0.08	***
number of female florets per capitulum	32-47	40.9 ± 4.7	29-43	36.5 ± 5.3	*ns*	36-48	41.9 ± 4.36	33-40	36 ± 2.71	**
number of hermaphrodite florets per capitulum	31-57	45.9 ± 9.4	22-42	30.9 ± 7.0	***	43-56	49.2 ± 4.32	42-53	47.4 ± 4.65	*ns*
total number of florets per capitulum	70-104	86.8 ± 13.8	51-80	67.4 ± 11.8	**	80-103	91.1 ± 8.45	76-92	83.4 ± 5.77	*ns*
female:hermaphrodite floret ratio	0.44-0.53	0.47 ± 0.03	0.49-0.6	0.55 ± 0.03	***	0.44-0.48	0.46 ± 0.01	0.38-0.47	0.43 ± 0.03	*
female-floret corolla tube length (mm)	2.4-2.6	2.52 ± 0.05	2.23-2.51	2.39 ± 0.1	**	2.25-2.35	2.30 ± 0.03	2.25-2.43	2.33 ± 0.07	*ns*
hermaphrodite-floret corolla tube length (mm)	2.95-3.05	3 ± 0.03	2.63-2.94	2.77 ± 0.11	***	2.58-2.75	2.66 ± 0.07	2.48-2.65	2.55 ± 0.05	**
female-floret pappus length (mm)	3-3.24	3.19 ± 0.08	2.87-3.09	2.97 ± 0.07	NT	3.2-3.36	3.28 ± 0.05	3.03-3.44	3.27 ± 0.12	*ns*
hermaphrodite-floret pappus length (mm)	3.24-3.4	3.31 ± 0.05	3.05-3.38	3.23 ± 0.12	*ns*	3.32-3.44	3.39 ± 0.04	3.28-3.48	3.37± 0.07	*ns*
involucral-bract lamina length (mm)	4.1-4.5	4.3 ± 0.13	3.64-4.36	3.93 ± 0.25	***	3.7-4.1	3.95 ± 0.11	3.3-3.6	3.45 ± 0.1	NT

Table 4.3. Comparison of continuous floral characters measured from cultivated and field-grown clones of the putative hybrids *W9* and *W10*. The means were calculated from up to ten measurements per clone. Significance levels in Student's *t* tests: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; *ns*, $P > 0.05$ (i.e., means not significantly different); NT, *t* tests not performed as data for at least one clone was non-normal, as indicated by normal probability plots.

Plate 2. Growth form of a putative hybrid between *Anaphalioides bellidioides* and *Ewartia sinclairii* and the gnaphalioid species growing at the Yeo Stream site. (**A–C** were photographed on 21 November 1989).

A, A putative hybrid between *A. bellidioides* and *E. sinclairii* growing at the study site (photo John Lovis).

B, *Anaphalioides bellidioides* and a putative hybrid with *Ewartia sinclairii* growing at the study site (photo John Lovis).

C, *Ewartia sinclairii* growing beside the Yeo Stream (photo John Lovis).

D, *Helichrysum coralloides* (photo Rainer Vogt).

E, *Helichrysum parvifolium*, upper Hodder valley, Inland Kaikoura Range.

F, *Ozothamnus leptophyllus*, Mt Robert Skifield Road, Travers Range (photo Ines Schönberger).


A
B
C
D
E
F

Table 4.4. Discrete characters recorded for all sympatric gnaphalioid species and the putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*. 'Field-collected putative hybrids' encompasses *all* putative hybrids found growing at the study site (i.e., cultivated *and* field-grown plants) except *W3*.

Key to characters: 1, growth form; 2, rooting pattern; 3, nonflowering shoot orientation; 4, morphologically distinct juvenile phase; 5, distinct internodes on flowering shoots; 10, leaf/stem angle; 11, leaf tip margins; 12, lamina margins; 13, shape of the lamina base; 14, stem enclosure by petiole extensions; 16, mucro orientation; 17, leaf indumentum density (adaxial surface); 18, leaf indumentum density (abaxial surface); 19; type B clothing trichomes on leaf; 20, type B glandular trichomes on leaf; 26, nerves raised on adaxial lamina surface; 27, midrib raised on abaxial leaf surface; 28, lateral nerves raised on abaxial leaf surface; 35, morphologically distinct flowering shoots; 36, transition from leaves to involucral bracts; 37, capitulum pedunculate; 47, receptacle type; 48, receptacle scales; 52, inner involucral bract, lamina colour; 53, lamina of inner involucral bracts hygroscopic; 54, inner involucral bract, shape of lamina tip; 55, inner involucral bract, gap colour; 59, upper corolla tube colour at anthesis; 60, corolla tube of outermost florets strongly curved; 61, corolla lobe colour at anthesis; 62, corolla lobes become crimson with age; 63, Corolla lobes recurved; 64, Crimson coloration in anthers; 65, Pollen colour; 66, Style arm colour; 68, Female floret pappus hairs, number of apical cells; 69, Female floret pappus hairs distinctly dimorphic; 70, Female floret pappus hairs, apical cells distinctly protruding; 72, Hermaphrodite floret pappus hairs, number of apical cells; 73, Pappus hairs, shape of apical cells; 74, Type of wall thickening in pappus hair apical cells; 85, Twin hairs on ovary of female florets; 86, Twin hairs on ovary of hermaphrodite florets; 87, Twin hairs, shape of terminal cells; 88, Multicellular twin hairs on ovary of female florets; 89, Multicellular twin hairs on ovary of hermaphrodite florets. NA = not applicable; ND = data missing.

Character	*A. bellidioides* (six plants)	*E. sinclairii* (nine plants)	*H. coralloides* (three plants)	*H. parvifolium* (three plants)	*O. leptophyllus* (five plants)	Field-collected putative hybrids	*W3*	*S1*	*S2* and *S3*
1	mat	subshrub	subshrub	shrub	shrub	mat	mat	mat	mat
2	nodal	basal	basal	basal	tap-rooted	nodal	nodal	nodal	nodal
3	prostrate	ascending or erect	erect	erect	erect	prostrate	prostrate	prostrate	prostrate
4	absent	absent	present	present	absent	absent	absent	absent	absent
5	present	present	absent	absent	present	present	present	present	present
10	± 90°	± 90°	< 20 °	< 20 °	± 90°	± 90°	± 90°	± 90°	± 90°
11	plane	plane	cucullate	cucullate	plane	plane	plane	plane	plane
12	plane	plane	involute	involute	plane or undulate	plane	plane	plane	plane
13	narrowed	tapering	tapering	tapering	tapering	narrowed or tapering	narrowed	narrowed	narrowed
14	> 50%	≤ 50%	≤ 50%	≤ 50%	≤ 50%	> 50%	> 50%	> 50%	> 50%
16	180 °	< 90 °	NA	NA	180 °	90–180 °	180 °	90–180 °	90–180 °
17	sparse	moderate-dense	dense	dense	sparse	moderate	sparse	moderate	sparse
18	dense	dense	sparse to glabrous	sparse to glabrous	dense	dense	dense	dense	dense
19	absent	absent	present	absent	absent	absent	absent	absent	absent
20	present	absent	absent	absent	absent	present	present	present	present
26	plane	plane	raised	raised	plane	plane	plane	plane	plane
27	raised	raised	plane	plane	raised	raised	raised	raised	raised
28	plane	raised	plane	plane	plane	raised	plane	plane	plane
35	present	absent	absent	absent	absent	present	present	present	present
36	gradual	abrupt	abrupt	abrupt	abrupt	gradual	gradual	gradual	gradual
37	pedunculate	pedunculate	sessile	sessile	pedunculate	pedunculate	pedunculate	pedunculate	pedunculate
47	scrobiculate	fimbrillate	fimbrillate	fimbrillate	fimbrillate	alveolate or foveolate	NA	scrobiculate	scrobiculate
48	absent	absent	absent	absent	present	absent	absent	absent	absent
52	white	white	pale yellow	pale yellow	white	white	white	white	white
53	present	present	absent	absent	present	present	present	present	present
54	acute to obtuse	rounded	acute to obtuse	acute to obtuse	rounded	acute to rounded	acute to obtuse	acute to obtuse	acute to rounded
55	straw	red-purple	straw	straw	straw	red-purple or straw	straw	straw	straw
59	pale green	crimson	pale green	pale green	pale green	pale green	ND	pale green	pale green
60	absent	absent	present	present	absent	absent	absent	absent	absent
61	pale green	white	bright yellow	bright yellow	white	greenish-white	ND	pale green	pale green

Table 4.4 (continued).

Character	*A. bellidioides* (six plants)	*E. sinclairii* (nine plants)	*H. coralloides* (three plants)	*H. parvifolium* (three plants)	*O. leptophyllus* (five plants)	Field-collected putative hybrids	*W3*	*S1*	*S2* and *S3*
62	present or absent	absent	absent	absent	absent	present or absent	present	absent	present or absent
63	erect	patent or recurved	patent or recurved	patent or recurved	patent or recurved	patent or recurved	erect	erect	patent or recurved
64	absent	dark crimson	absent	absent	absent	pale crimson	absent	absent	absent
65	yellow	white	yellow	yellow	pale yellow	pale yellow or white	ND	pale yellow	yellow
66	pale green	white	yellow	yellow	white	greenish-white	ND	greenish-white	pale green
68	1–2	3–6	2–3	1–3	NA	1–3	1–2	1–3	1–3
69	absent	present	absent	absent	NA	absent	absent	absent	absent
70	absent	present	absent	absent	NA	absent or present	absent	absent	absent
72	1–2	5–8	3–4	1–3	1–5	3–5	1–2	3–5	3–5
73	clavate	clavate	acute	acute	clavate	clavate	acute	clavate	clavate
74	uniform	reticulate	pitted	uniform	reticulate	reticulate	uniform	reticulate	reticulate
85	absent	absent or present	present	present	NA	absent or present	absent	present	absent or present
86	absent	absent	present	present	present	absent	absent	present	absent
87	NA	clavate	acute	acute	clavate	clavate	NA	clavate	clavate
88	absent	absent	absent	absent	absent	absent	absent	present	absent
89	absent	absent	absent	absent	present	absent	absent	absent	absent

Table 4.5. Continuous characters recorded from field-grown specimens of all sympatric gnaphalioid species and putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*. The range, mean and standard deviation are presented for each character. (continued overleaf)

Key to characters: 6, leaf length (mm); 7, maximum lamina width (mm); 8, leaf length: lamina width ratio; 9, point of maximum lamina width: leaf length ratio; 15, mucro length (mm); 38, number of capitula per inflorescence; 39, capitulum length (mm); 40, capitulum width at midpoint (mm); 41, number of female florets per capitulum; 42, number of hermaphrodite florets per capitulum; 43, total number of florets per capitulum; 44, female: hermaphrodite floret ratio; 45, receptacle height (mm); 46, receptacle diameter (mm); 49, inner involucral bract length (mm); 50, inner involucral bract, lamina length (mm); 51, inner involucral bract, lamina width (mm); 57, corolla tube length in female florets (mm); 58, corolla tube length in hermaphrodite florets (mm); 67, pappus hair length in female florets (mm); 71, pappus hair length in hermaphrodite florets (mm); 78, female floret ovary length (mm); 79, female floret ovary width (mm); 80, female floret ovary length: width ratio; 81, hermaphrodite floret ovary length (mm); 82, hermaphrodite floret ovary width (mm); 83, hermaphrodite floret ovary length:width ratio. NA = character not applicable; ND = data missing.

Species / putative hybrid	Character																	
	6	7	8	9	15	38	39	40	41	42	43	44	45	46	49	50	51	57
A. bellidioides (six plants)	3.2-7.5 5.2 ± 1.0	1.5-2.9 2.3 ± 0.3	1.6-2.9 2.3 ± 0.3	0.23-0.39 0.30 ± 0.05	0.13-0.38 0.26 ± 0.05	1	6.5-8.2 7.5 ± 0.6	5.4-7.7 6.7 ± 0.7	78-117 95.4 ± 11.7	68-111 86.4 ± 11.2	147-228 181.9 ± 21.9	0.49-0.57 0.52 ± 0.02	1.9-3 2.3 ± 0.3	2-2.6 2.3 ± 0.2	7.2-9.7 8.3 ± 0.6	4.5-6 5.2 ± 0.4	1.1-1.7 1.4 ± 0.2	2.2-2.8 2.5 ± 0.2
E. sinclairii (nine plants)	6.8-15.7 11.1 ± 2.2	1.5-4.2 2.8 ± 0.6	3.3-5.6 4.1 ± 0.4	0.18-0.45 0.32 ± 0.06	0.08-0.2 0.11 ± 0.1	6-35 21.3 ± 8.3	4.3-5.8 4.9 ± 0.3	2.1-3.1 2.6 ± 0.2	6-14 9.4 ± 1.6	12-25 17.8 ± 3.1	18-39 27.2 ± 4.2	0.27-0.45 0.35 ± 0.04	0-0.3 0.12 ± 0.11	1.3-1.8 1.5 ± 1.4	3.5-4.8 4.2 ± 0.3	1.5-2.3 1.9 ± 0.2	0.9-1.5 1.2 ± 0.1	1.8-2.6 2.1 ± 0.2
H. coralloides (three plants)	4.5-6.2 5.3 ± 0.4	2.6-3.8 3.2 ± 0.3	1.3-1.9 1.7 ± 0.1	0.58-0.94 0.75 ± 0.08	0 0	1	6.6-7.9 7.2 ± 0.4	3.6-4.8 4.2 ± 0.4	10-31 19.6 ± 6.1	40-81 58.4 ± 11.7	51-112 77.9 ± 17.1	0.18-0.32 0.25 ± 0.04	0.3-0.8 0.52 ± 0.1	1.4-2.6 2.1 ± 0.4	5-6.4 5.7 ± 0.4	ND	1.1-1.6 1.3 ± 0.1	3.2-4 3.5 ± 0.2
H. parvifolium (three plants)	1.4-1.87 1.6 ± 0.1	1-1.2 1.1 ± 0.1	1.3-1.6 1.5 ± 0.1	0.84-0.95 0.91 ± 0.03	0.03-0.08 0.04 ± 0.01	1	5-6.1 5.5 ± 0.3	2.5-3 2.8 ± 0.2	5-12 7.9 ± 2.2	19-34 27.3 ± 3.3	27-40 35.2 ± 3.5	0.14-0.31 0.23 ± 0.06	0.2-0.4 0.31 ± 0.05	1.2-1.6 1.4 ± 0.1	3.6-4.4 4.0 ± 0.2	ND	1.1-1.6 1.3 ± 0.1	2.4-3.2 2.8 ± 0.2
O. leptophyllus (five plants)	6.5-9.1 7.7 ± 0.8	2-3.4 2.6 ± 0.4	2.2-3.5 2.9 ± 0.3	0.23-0.44 0.33 ± 0.05	0-0.14 0.04 ± 0.05	14-61 30.5 ± 12.2	3.8-5.2 4.6 ± 0.3	1.8-2.8 2.2 ± 0.2	0	9-20 12 ± 3.1	9-20 12 ± 3.1	0	0.2-0.5 0.31 ± 0.1	0.6-1.1 0.8 ± 0.2	3.2-4.4 3.9 ± 0.4	0.7-1.2 1.0 ± 0.1	0.8-1.5 1.1 ± 0.1	NA
W4	4.4-6 5.1 ± 0.6	1.7-2.4 2.0 ± 0.2	2.3-2.8 2.6 ± 0.2	0.21-0.28 0.26 ± 0.02	0.28-0.41 0.34 ± 0.05	4	5.7	3.9	39	39	78	0.5	0.7	1.6	5.6-6.2 6.0 ± 0.2	3.2-3.6 3.4 ± 0.2	0.7-1 0.9 ± 0.1	2.1-2.3 2.2 ± 0.1
W5	ND	ND	ND	ND	ND	1-5	6.1	4.7	37	43	80	0.46	0.5	1.8	5.7-6.2 6.0 ± 0.2	2.9-3.5 3.2 ± 0.2	1-1.8 1.4 ± 0.3	2.3-2.6 2.4 ± 0.1
W8	5.4-6 5.6 ± 0.2	2.1-2.4 2.3 ± 0.1	2.3-2.8 2.5 ± 0.2	0.25-0.33 0.29 ± 0.03	0.13-0.25 0.2 ± 0.05	4	6.1	3.8	40	42	82	0.49	0.7	1.8	5.7-6.2 6.0 ± 0.2	3.1-3.7 3.4 ± 0.2	1-1.4 1.2 ± 0.1	2.2-2.3 2.2 ± 0.03
W9	6.5-9 7.6 ± 0.8	2.9-3.5 3.2 ± 0.2	2.2-2.7 2.4 ± 0.2	0.24-0.32 0.28 ± 0.03	0.15-0.23 0.19 ± 0.03	3-6 3.9 ± 1.1	5.8-6.6 6.1 ± 0.2	3.2-4.2 3.7 ± 0.5	29-43 36.5 ± 5.3	22-42 30.9 ± 7.0	51-85 67.4 ± 11.8	0.49-0.6 0.55 ± 0.03	0.7-0.9 0.74 ± 0.07	1.4-1.9 1.6 ± 0.1	6.2-7.2 6.7 ± 0.3	3.6-4.3 3.9 ± 0.3	1.1-1.4 1.2 ± 0.1	2.2-2.5 2.4 ± 0.1
W10	5.6-6.9 6.2 ± 0.4	1.9-2.6 2.3 ± 0.2	2.5-3.2 2.8 ± 0.2	0.25-0.36 0.29 ± 0.03	0.2-0.28 0.24 ± 0.03	1-3 1.9 ± 0.9	6.1-6.6 6.3 ± 0.2	4-4.5 4.2 ± 0.2	33-40 36 ± 2.7	42-53 47.4 ± 4.7	76-92 83.4 ± 5.8	0.38-0.47 0.43 ± 0.03	0.9-1.1 0.94 ± 0.09	1.5-1.8 1.6 ± 0.1	5.8-6.2 6.0 ± 0.1	3.3-3.6 3.5 ± 0.1	1-1.1 1.0 ± 0.04	2.3-2.4 2.3 ± 0.1
W12	3.2-4.8 3.8 ± 0.6	1.3-2 1.6 ± 0.2	2.1-2.6 2.3 ± 0.2	0.32-0.38 0.36 ± 0.02	0.15-0.28 0.19 ± 0.04	1	ND	ND	22	28	50	0.44	1.2	1.9	5-5.6 5.3 ± 0.3	3.1-3.5 3.4 ± 0.2	0.8-0.9 0.9 ± 0.03	2.6-2.7 2.7 ± 0.03

Table 4.5 (continued).

Species / putative hybrid	Character								
	58	67	71	78	79	80	81	82	83
A. bellidioides (six plants)	2.2-2.9 2.6 ± 0.2	2.8-3.8 3.3 ± 0.3	3.1-4 3.5 ± 0.2	0.6-0.9 0.7 ± 0.1	0.18-0.26 0.23 ± 0.02	2.6-3.9 3.1 ± 0.3	0.6-0.9 0.7 ± 0.1	0.2-0.33 0.29 ± 0.02	1.9-3.4 2.5 ± 0.3
E. sinclairii (nine plants)	2-2.8 2.3 ± 0.2	2-2.9 2.5 ± 0.2	2.2-3 2.6 ± 0.2	0.6-1 0.8 ± 0.1	0.26-0.4 0.32 ± 0.03	1.5-3.3 2.4 ± 0.4	0.6-1 0.8 ± 0.1	0.25-0.4 0.33 ± 0.03	1.8-3 2.4 ± 0.3
H. coralloides (three plants)	3.3-4.3 3.8 ± 0.3	3.4-4 3.7 ± 0.2	3.4-4.2 4.0 ± 0.2	0.8-1.4 1.2 ± 0.2	0.26-0.38 0.32 ± 0.03	2.5-5.4 3.8 ± 0.7	0.9-1.4 1.2 ± 0.1	0.26-0.4 0.34 ± 0.03	2.5-4.4 3.4 ± 0.5
H. parvifolium (three plants)	3-3.4 3.2 ± 0.1	2.6-3.4 3.0 ± 0.3	2.7-3.6 3.1 ± 0.3	1-1.3 1.1 ± 0.1	0.29-0.38 0.32 ± 0.02	2.8-4.2 3.5 ± 0.3	0.8-1.3 1.1 ± 0.1	0.28-0.38 0.34 ± 0.03	2.4-3.9 3.3 ± 0.4
O. leptophyllus (five plants)	2.2-2.9 2.5 ± 0.2	NA	2.4-3.3 2.9 ± 0.2	NA	NA	NA	0.7-1 0.8 ± 0.1	0.3-0.5 0.41 ± 0.05	1.7-3.2 2.1 ± 0.3
W4	2.5-2.7 2.6 ± 0.1	2.6-3 2.8 ± 0.2	2.9-3 3.0 ± 0.1	0.55-0.65 0.58 ± 0.04	0.15-0.23 0.18 ± 0.03	2.7-3.9 3.3 ± 0.4	0.53-0.63 0.57 ± 0.04	0.18-0.23 0.2 ± 0.02	2.5-3.2 2.9 ± 0.3
W5	2.7-2.8 2.7 ± 0.1	3.2-3.5 3.3 ± 0.1	3.4-3.7 3.6 ± 0.1	0.65-0.7 0.69 ± 0.02	0.16-0.2 0.19 ± 0.01	3.3-4.4 3.7 ± 0.3	0.6-0.7 0.64 ± 0.03	0.18-0.23 0.2 ± 0.02	2.6-3.8 3.2 ± 0.4
W8	2.5-2.7 2.6 ± 0.1	2.8-2.9 2.8 ± 0.1	3.1-3.2 3.2 ± 0.04	0.58-0.63 0.6 ± 0.02	0.14-0.2 0.17 ± 0.02	2.9-4.3 3.5 ± 0.5	0.48-0.65 0.56 ± 0.05	0.16-0.23 0.19 ± 0.02	2.6-3.3 2.9 ± 0.2
W9	2.6-2.9 2.8 ± 0.1	2.9-3.1 3.0 ± 0.1	3.1-3.4 3.2 ± 0.1	0.53-0.68 0.61 ± 0.05	0.23-0.28 0.25 ± 0.01	2.1-2.7 2.4 ± 0.2	0.63-0.75 0.66 ± 0.05	0.25-0.31 0.28 ± 0.02	2.2-2.8 2.4 ± 0.2
W10	2.5-2.7 2.6 ± 0.1	3-3.4 3.3 ± 0.1	3.3-3.5 3.4 ± 0.1	0.43-0.55 0.5 ± 0.04	0.24-0.28 0.26 ± 0.01	1.7-2.1 1.9 ± 0.1	0.65-0.85 0.76 ± 0.06	0.24-0.3 0.27 ± 0.02	2.3-3.3 2.9 ± 0.3
W12	2.8-3.1 2.9 ± 0.1	3.5-3.6 3.6 ± 0.1	3.4-3.6 3.6 ± 0.1	0.8-0.95 0.89 ± 0.05	0.2-0.29 0.26 ± 0.03	2.9-4.7 3.5 ± 0.6	0.88-0.98 0.93 ± 0.07	0.26-0.31 0.29 ± 0.04	3.2-3.4 3.3 ± 0.2

Species / putative hybrid	Character			
	6	7	8	15
A. bellidioides (six plants)	12.7-15.5 14.0 ± 0.6	4.9-6.3 5.5 ± 0.3	2.3-2.8 2.5 ± 0.1	0.35-0.45 0.39 ± 0.02
E. sinclairii (five plants)	17.6-22.4 20.0 ± 1.2	5.4-7 6.3 ± 0.5	2.6-3.6 3.2 ± 0.2	0.15-0.2 0.17 ± 0.02
H. coralloides (six plants)	5.5-9 7.4 ± 1.1	2.8-4.4 3.6 ± 0.4	1.4-3.1 2.1 ± 0.4	0 0
H. parvifolium (four plants)	2.6-3.6 2.9 ± 0.2	1-1.4 1.1 ± 0.1	2-3.6 2.6 ± 0.4	0 0
O. leptophyllus (three plants)	9.6-12.3 11.2 ± 1.0	2.9-4 3.3 ± 0.3	2.9-4.1 3.5 ± 0.4	0.05-0.18 0.10 ± 0.04
W1	14.6-17.6 16.2 ± 0.8	4.5-5.5 5.0 ± 0.3	3.1-3.4 3.2 ± 0.04	0.25-0.38 0.30 ± 0.01
W2	17.8-19.6 18.7 ± 0.6	5.8-6.7 6.2 ± 0.3	2.9-3.1 3.0 ± 0.04	0.28-0.38 0.32 ± 0.01
W9	16-18.1 17.1 ± 0.7	5.8-6.5 6.2 ± 0.2	2.6-2.9 2.8 ± 0.1	0.4-0.48 0.44 ± 0.02
W10	13.8-15.2 14.1 ± 0.6	4.4-5.2 4.8 ± 0.2	2.8-3.1 2.9 ± 0.1	0.33-0.38 0.35 ± 0.02
W11	16.3-20.8 18.9 ± 1.3	6.5-7.3 6.9 ± 0.3	2.5-3 2.7 ± 0.2	0.38-0.48 0.44 ± 0.03
W13	18.1-19.8 18.9 ± 0.5	5.8-6.5 6.2 ± 0.2	2.9-3.2 3.1 ± 0.1	0.23-0.3 0.27 ± 0.02
S1	15-16.2 15.5 ± 0.4	5.5-6 5.8 ± 0.2	2.3-2.6 2.5 ± 0.1	0.2-0.28 0.25 ± 0.03
S2	14.1-15 14.6 ± 0.3	4.9-5.5 5.2 ± 0.2	2.7-2.9 2.8 ± 0.1	0.38-0.45 0.42 ± 0.02
S3	14.3-15.1 14.7 ± 0.2	5-5.6 5.3 ± 0.2	2.7-2.9 2.8 ± 0.1	0.35-0.43 0.40 ± 0.02

Table 4.6. Comparison of continuous vegetative characters recorded from cultivated plants of all sympatric species and putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*. The range, mean and standard deviation are presented for each character. Key to characters: 6, leaf length (mm); 7, maximum lamina width (mm); 8, leaf length: lamina width ratio; 15, mucro length (mm).

4.3.3.3 Analyses of morphological data

Canonical discriminant analysis

Normal probability plots indicated five transformed characters (9, 38, 41, 45 and 49) were still not normally distributed. Box plots indicated the distribution of character 38 was strongly skewed and that outliers were present in characters 9, 41, 45, 50, 58 and 81.

Unless stated otherwise, in all analyses Box's M and adjusted M tests indicated high homogeneity of covariances ($P > 0.95$). Wilk's lambda, Pillai's trace and Roy's greatest root (all $P < 0.001$) indicated the group means were significantly different. Only in the analysis of all 20 available characters was the Hotelling-Lawley trace not significant ($P = 0.0727$). Hotelling's T^2 test indicated the group means were significantly different in all analyses. The canonical variates were never significantly correlated ($P < 0.001$). All OTUs of the species were correctly classified by plug-in classification and the rule mean squared error was zero.

In the analysis of 20 characters, each canonical variate explained 69.8 %, 13.7 %, 11.1 % and 5.3 % of the total variation. The results of jackknifing and the posterior probability of group membership error rates for each group are summarised in Table 4.7. On scatter plots of the first, second and third canonical variates, each species formed tight, well-separated clusters (Figure 4.1 A–C p. 118). The first canonical variate principally discriminated *A. bellidioides*, *H. parvifolium* and the putative hybrids, while *A. bellidioides*, *E. sinclairii* and the putative hybrids were clustered on the second and third canonical variates. The putative hybrids (*W4*, *W8*, *W9* and *W10*) were placed between *A. bellidioides* and *E. sinclairii* on the first axis, but did not form a distinct group. *W9* and *W10* were equidistant between *A. bellidioides* and *E. sinclairii*, whereas *W4* and *W8* were closer to *E. sinclairii*. On the third canonical variate, *W9* clustered with the putative parental species, but *W4*, *W8* and *W10* were separated from this

Species	*A. bellidioides*	*E. sinclairii*	*H. coralloides*	*H. parvifolium*	*O. leptophyllus*	Error	Posterior error
A. bellidioides	6	0	0	0	0	0	0
E. sinclairii	0	9	0	0	0	0	0
H. coralloides	0	0	2	0	1	0.33	0.33
H. parvifolium	0	0	1	2	0	0.33	0
O. leptophyllus	0	1	0	0	4	0.2	-0.11
overall						0.1154	0

Table 4.7. Cross-validation table for the canonical discriminant analysis of 20 continuous characters recorded from field-grown plants. Values represent the number of OTUs classified per group and misclassification error rates.

group with intermediacy between *H. coralloides* and the putative parents suggested. The number of capitula per inflorescence was highly correlated with the first axis, but most characters contributed more or less equally to the first and second canonical variates (Figure 4.2 A p. 119). The Pearson's product-moment correlation coefficients between each character and the first three canonical variates are summarised in Table 4.8 (p. 120). With plug-in classification, *W4*, *W8* and *W9* were predicted to belong to *A. bellidioides* ($P = 1$) and *W10* to *E. sinclairii* ($P = 1$).

When characters containing outliers were excluded, each canonical variate accounted for 69.6 %, 18.5 %, 10.1 % and 1.7 % of the total variation. One plant of *H. coralloides* was misclassified as *H. parvifolium* by jackknifing. The placement of the putative hybrids in relation to the species was similar to the initial analysis. However, although still close to the other putative hybrids, *W10* was placed intermediate between *A. bellidioides* and either *H. parvifolium* or *O. leptophyllus* on plots of the first, second and third canonical variates (Figure 4.3 A–C p. 121). On the second and third axes *W4*, *W8* and *W10* were intermediate between the *A. bellidioides–E. sinclairii–W9* cluster and *H. parvifolium*, and *W10* was placed close to *O. leptophyllus*. The characters contributing most to the first canonical variate differed from the initial analysis. Involucral bract length, capitulum width, total floret number per capitulum and hermaphrodite floret ovary width were highly correlated with the first axis (Figure 4.2 B p. 119). No characters were strongly correlated with the second canonical variate. Plug-in classification generated identical predictions of group membership to the initial analysis.

To allow the inclusion of *W5* and *W12* in the analysis, characters for which data were missing (6, 7, 8, 9, 15, 39 and 40) were excluded from the original data set. The proportion of the total variation explained by each canonical variate was 79.6 %, 12.6 %, 6.3 % and 1.4 %. One plant of *H. coralloides* was incorrectly classified as *H. parvifolium* with jackknifing. On scatter plots of the canonical variates, the species were well separated but formed looser clusters than in the previous analyses and the putative hybrids were more widely dispersed. The first canonical variate principally discriminated *A. bellidioides* and the putative hybrids from the other species, The putative hybrids were placed between *A. bellidioides* and the other species on the first axis and did not form a distinct cluster. On the first and second canonical variates *W4*, *W5*, *W8* and *W9* were intermediate between *A. bellidioides* and *E. sinclairii*. *W10* and *W12* were separated from the other putative hybrids. Groups were less clearly differentiated on the third axis, with *A. bellidioides*, *E. sinclairii*, *H. parvifolium* and the putative hybrids forming a large cluster. Six of the 13 characters analysed were highly (and

negatively) correlated with the first axis (Figure 4.4 A p. 122). The best discriminating characters on the first axis were floret numbers per capitulum, receptacle dimensions and involucral bract length. Corolla tube length of hermaphrodite florets was the only character strongly correlated with the second canonical variate. With plug-in classification, all putative hybrids were predicted to belong to *A. bellidioides* ($P = 1$) except *W12*, which was classified as *H. parvifolium* ($P = 1$).

Characters containing outliers were excluded and the eight remaining characters reanalysed. Each canonical variate explained 86.5 %, 10.7 %, 1.7 % and 1.2 % of the total variation. One plant of *H. coralloides* was incorrectly classified as *H. parvifolium* with jackknifing. The species still formed loose clusters in scatter plots of the canonical variates, but the groups were clearly separated (Figure 4.5 A–C p. 123). The position of the species on the axes was generally unchanged but placement of the putative hybrids often differed. All putative hybrids were intermediate between *A. bellidioides* and *E. sinclairii* on the first canonical variate, and both species and the putative hybrids formed a loose grouping on the second and third canonical variates. *W4* and *W9* were always intermediate between the putative parental species. *W5* and *W8* were relatively close on each axis but were somewhat isolated on the third axis. was closest to *W10* and both were represented as intermediate between *A. bellidioides* and *H. coralloides* or *H. parvifolium*. *W12* was closest to *W5* but was only intermediate between *A. bellidioides* and *E. sinclairii* on the first axis. The second and third canonical variates suggested *W12* was closer to *H. parvifolium*. *W10* was close to *W4* and *W9* on the first and third axes, but was somewhat isolated on the second axis. Character correlations for the first and second axes were little changed from the previous analysis (Figure 4.4 B p. 122). Results of plug-in classification were similar to the previous analysis, but *W10* received a low probability of membership to *H. parvifolium* ($P = 0.093$).

Since discrete characters suggested *H. coralloides* and *H. parvifolium* were the least likely parents, these species were excluded and the data reanalysed. Only two canonical variates were generated in these analyses. To enable simultaneous comparison of all hybrids, characters containing outliers or missing data were excluded, leaving eight characters in the data set. The canonical variates explained 94.4 % and 5.6 % of the variation. All putative hybrids were intermediate between *A. bellidioides* and *E. sinclairii* on the first canonical variate (Figure 4.6 p. 123). *W12* was considerably closer to *E. sinclairii* than the other putative hybrids, which were essentially equidistant between the two species. The second

canonical variate principally accounted for variation among the putative hybrids. The placement of *W9* and *W12* was identical to the first canonical variate, *W4* and *W8* were placed within the *A. bellidioides* group, and *W5* and *W10* were placed close to or within the *O. leptophyllus* group. All characters except pappus-hair length in hermaphrodite florets were strongly correlated with the first canonical variate (Figure 4.7 p. 124). When characters containing outliers were included in the analysis, Box's *M* test indicated covariance heterogeneity was highly significant ($P = 0$), but in an adjusted *M* test the difference was highly insignificant ($P = 1$).

HYWIN

With 32 OTUs in each data set analysed, 14 880 hypotheses were generated of which the 201 highest-ranked combinations (representing the 0.95 probability that each OTU would be ranked as a hybrid at least once) were considered.

Continuous characters were first analysed with the default weightings (wI=1, wE=1, wP=1). The six putative hybrids were hypothesised to be hybrids within the 40 highest-ranked combinations. *W8*, *W9* and *W10* were the highest and most frequently ranked as hybrids. Plants of *H. parvifolium* and *E. sinclairii* were hypothesised as hybrids, and *W5* (three times) and *W8* (once) were ranked as parents, in the 201 highest rankings. Of the 20 highest-ranked combinations, three putative hybrids (*W8*, *W9* and *W10*) were hypothesised to be *A. bellidioides* × *O. leptophyllus* 12 times, *W8* and *W9* were ranked as *A. bellidioides* × *E. sinclairii* four times, and *W12* was twice ranked as *A. bellidioides* × *H. parvifolium*. Of the 100 highest-ranked combinations, putative hybrids were most frequently hypothesised to be *A. bellidioides* × *O. leptophyllus* (58 times) and *A. bellidioides* × *E. sinclairii* (20 times). *W4*, *W8*, *W9* and *W10* were predominantly hypothesised to be *A. bellidioides* × *O. leptophyllus*; *W5* was most frequently ranked as *E. sinclairii* × *H. coralloides*; and *W12* was only ranked as *A. bellidioides* × *H. parvifolium* or *E. sinclairii* × *H. coralloides*.

When the intermediacy weighting was low (wI=0.1, wE=1, wP=1), OTUs of the species were more frequently hypothesised as hybrids. All of the putative hybrids were hypothesised to be hybrids within the 40 highest rankings, but none were hypothesised to be parents in the 201 highest rankings. The putative hybrids were predominantly hypothesised to be *A. bellidioides* × *O. leptophyllus*; only eight times in the 100 highest rankings was either *W4*, *W8*, *W9* or *W10* hypothesised to be *A. bellidioides* × *E. sinclairii*.

When equality received a low weighting (wI=1, wE=0.1, wP=1), fewer species OTUs were hypothesised to hybrids and *W5* was hypothesised to be a parent six times. Among the 100 highest rankings, the putative hybrids were predominantly hypothesised to be *A. bellidioides* × *O. leptophyllus*. *W8* and *W9* were the most frequently ranked as *A. bellidioides* × *E. sinclairii* (four and six times respectively). *W12* was only hypothesised to be *A. bellidioides* × *H. parvifolium*. Similar results were obtained when the parental-distance weighting was low (wI=1, wE=1, wP=0.1).

All but one OTU was hypothesised to be a hybrid within the 201 highest rankings with low intermediacy and equality weightings (wI=0.1, wE=0.1, wP=1). All of the putative hybrids were ranked as hybrids within the 13 highest rankings. Among the 100 highest-ranked combinations, the putative hybrids were hypothesised to be either *A. bellidioides* × *O. leptophyllus* (most frequently) or *E. sinclairii* × *H. coralloides* (infrequently).

In an analysis of discrete characters with the default weightings, the first 54 rankings were filled by different combinations hypothesising *W8* to be *A. bellidioides* × *E. sinclairii*, each with an identical hybrid optimality score. This pattern was repeated in lower-ranked combinations. In each instance, *A. bellidioides* and *E. sinclairii* were the hypothesised parents of the putative hybrids.

When the mixed data set was analysed with the default weightings, the putative hybrids *W4*, *W5*, *W8* and *W9* were hypothesised to be *A. bellidioides* × *E. sinclairii* without exception for the 137 highest-ranked combinations. Alternative parentage hypotheses for these plants (mainly *H. coralloides* × *O. leptophyllus* and *E. sinclairii* × *O. leptophyllus*) were occasionally ranked among the 201 most likely hypotheses. *W10* was first ranked as a hybrid at 166 and *W12* first ranked at 201. Both were hypothesised to be *A. bellidioides* × *E. sinclairii* only. A low parental-distance weighting (wI=1, wE=1, wP=0.1) had little impact, except *W10* and *W12* were not hypothesised as hybrids among the 201 highest-ranked triplets. With moderate intermediacy and equality weightings (wI=0.5, wE=0.5, wP=1), alternative parentage hypotheses were higher ranked, but *A. bellidioides* × *E. sinclairii* hypotheses still predominated. Low intermediacy and equality weightings (wI=0.1, wE=0.1, wP=1) resulted in a high frequency of species OTUs being ranked highly as possible hybrids. Among the 100 highest-ranked hypotheses, only *A. bellidioides* × *H. parvifolium* or *E. sinclairii* × *H. coralloides* were ranked as parentage hypotheses for the putative hybrids. Only four OTUs

(the three *H. coralloides* plants and one *H. parvifolium* plant) were not ranked as hybrids in the 201 highest-ranked combinations. Of the putative hybrids, *W5* and *W12* were the highest and most frequently ranked as hybrids.

Metric multidimensional scaling

Analysis of dissimilarities calculated from mixed data with all sympatric gnaphalioid species included yielded 17 positive eigenvalues. The first three principal coordinates accounted for 45.9 %, 34.3 % and 11.8 % of the total variation. The proportion of the total sum of squared distances for each axis was 0.62, 0.34 and 0.04 respectively. The first axis principally separated *H. coralloides* and *H. parvifolium* from the remaining OTUs (Figure 4.8 A & B p. 125). The second axis separated *A. bellidioides*, *E. sinclairii*, *O. leptophyllus* and the putative hybrids into groups (Figure 4.8 A & C). *H. coralloides* and *H. parvifolium* were clearly differentiated only on the third axis, on which *O. leptophyllus* was isolated. On scatter plots of the first and second principal coordinates, the putative hybrids formed a loose but well-separated group intermediate between *A. bellidioides* and either *E. sinclairii* or *O. leptophyllus*. On the third axis *W4*, *W8*, *W9* and *W10* were placed close to *E. sinclairii* and *H. coralloides*, but *W5* and *W12* formed a pairing isolated from all other OTUs. The putative hybrids *W4*, *W8*, *W9* and *W10* were intermediate between *A. bellidioides* and *E. sinclairii* on all scatter plots of the three axes; they were intermediate between *A. bellidioides* and *O. leptophyllus* on the first and second axes only, between *E. sinclairii* and *O. leptophyllus* on plots of the first and third axes, and between *A. bellidioides* and *H. coralloides* on the second and third axes only. Construction of minimal spanning trees on the principal-coordinate scatter plots indicated the putative hybrids were closer to *O. leptophyllus* than *E. sinclairii* on the first and second axes (Figure 4.9 A–C p. 126).

The first three principal coordinates derived from discrete characters explained 52.3 %, 28.8 % and 12.7 % of the total variation. The proportion of the total sum of squared distances for each axis was 0.73, 0.22 and 0.05 respectively. All species were well separated with little variation among individuals within each group (Figure 4.10 A & B p. 127). The putative hybrids formed a loose cluster on a scatter plot of the first and second axes and were intermediate between *A. bellidioides* and either *E. sinclairii* or *O. leptophyllus*. *W5* and *W12* were separated from all other OTUs on the third axis. The other putative hybrids clustered with *E. sinclairii* on scatter plots of the first and third axes, and were intermediate between *A. bellidioides* and either *E. sinclairii* or *H. coralloides* on plots of the second and third axes.

The first three principal coordinates derived from continuous characters accounted for 52.4 %, 24.6 % and 4.4 % of the total variation. The proportion of the total sum of squared distances for each axis was 0.81, 0.18 and 0.01 respectively. Greater within-group variation was evident in scatter plots of the first three axes and groups were less well defined by the third principal coordinate (Figure 4.10 C & D p. 127). *W4*, *W8*, *W9* and *W10* formed a tight cluster and were intermediate between *A. bellidioides* and *E. sinclairii* on a plot of the first and second axes. *W5* and *W12* were slightly separated from the other putative hybrids. The putative hybrids were widely spaced on the third principal coordinate.

Helichrysum coralloides and *H. parvifolium* were excluded and the mixed data set reanalysed. The first three axes accounted for 66 %, 21.7 % and 2.3 % of the total variation. On a scatter plot of the first and second principal coordinates, the remaining species were widely separated and four of the putative hybrids (*W4*, *W8*, *W9* and *W10*) were intermediate between *A. bellidioides* and *E. sinclairii*. *W5* and *W12* were slightly separated but closest to the other putative hybrids. There was no suggestion that *O. leptophyllus* was a possible parent. The putative hybrids clustered with *E. sinclairii* on the second axis. No groups were differentiated on the third axis, and *W12* was notably isolated from all other OTUs. When *E. sinclairii* and *O. leptophyllus* were excluded from the mixed data set, there was no suggestion of intermediacy of the putative hybrids between *A. bellidioides* and either *Helichrysum* species on the first three principal coordinates.

Split decomposition

An analysis of the dissimilarities with all sympatric gnaphalioid species included yielded 64 weakly compatible splits. The splits graph had a fit of 61.6 % (Figure 4.11 A & B p. 128). Each species was separated by internal edges except for *H. coralloides* and *H. parvifolium*. The putative hybrids formed a group near the centre of the splits graph between *A. bellidioides* and *Ewartia sinclairii*, but lacked an internal edge distinguishing the group. Comparatively long internal edges separated *H. coralloides*, *H. parvifolium* and *O. leptophyllus* from the other individuals. *W5* was slightly closer to *A. bellidioides* but the other putative hybrids were represented as being extremely similar. A Buneman tree, comprising 41 compatible split systems, had a fit of 46.6 %. Compatible splits separated each species and the group of putative hybrids.

Species were sequentially excluded from the data set and dissimilarities recalculated to assess the impact on the structure of the splits graphs and whether placement of the putative hybrids

in relation to the putative parental and non-parental species varied. In all analyses including *A. bellidioides*, the putative hybrids were most similar to *A. bellidioides*. In splits graphs the putative hybrids were always placed between *A. bellidioides* and the other species (Figure 4.11 C–E). When *A. bellidioides* and *O. leptophyllus* were included in the data set the putative hybrids group was distinguished by a compatible split (Figure 4.11 D). When either *E. sinclairii* or the *Helichrysum* species were included in the data set with *A. bellidioides*, the putative hybrids were placed intermediate between *A. bellidioides* and the other species, but the putative hybrids did not form a distinct group separated by an internal edge. Each putative hybrid was separated by a weakly compatible split. *W9* and *W10* were usually slightly closer to *A. bellidioides* than the other putative hybrids. *W4*, *W5* and *W8* were the most intermediate of the putative hybrids, but *W12* was often separated from the other putative hybrids and linked to the other species by a weakly compatible split. The fit in these analyses ranged from 68.3 to 82.4 %.

When *A. bellidioides* and *E. sinclairii* were excluded from the data set, a splits graph containing 46 weakly compatible split systems and a fit of 91.9 % was generated. A compatible split separated the putative-hybrids group from the other specimens. Three contradictory splits linked the *Helichrysum* species with the other specimens. When *A. bellidioides* and *O. leptophyllus* were excluded, a splits graph of similar structure and a fit of 90.7 % was generated. Four contradictory splits linked the putative hybrids and the *Helichrysum* species, but a single compatible split linked *E. sinclairii* with the putative hybrids. The putative-hybrids group was distinguished by weakly compatible splits only.

When only the putative hybrids were included in the analysis, a splits graph containing 11 weakly compatible splits and with a fit of 87 % was generated (Figure 4.11 F). The putative hybrids were again represented as very similar with no distinct groups. *W12* had the longest terminal edge and was thus represented as the most divergent specimen. There was some support for a close relationship between *W5* and *W12*, and between *W9* and *W10*, but all internal edges represented weakly compatible splits.

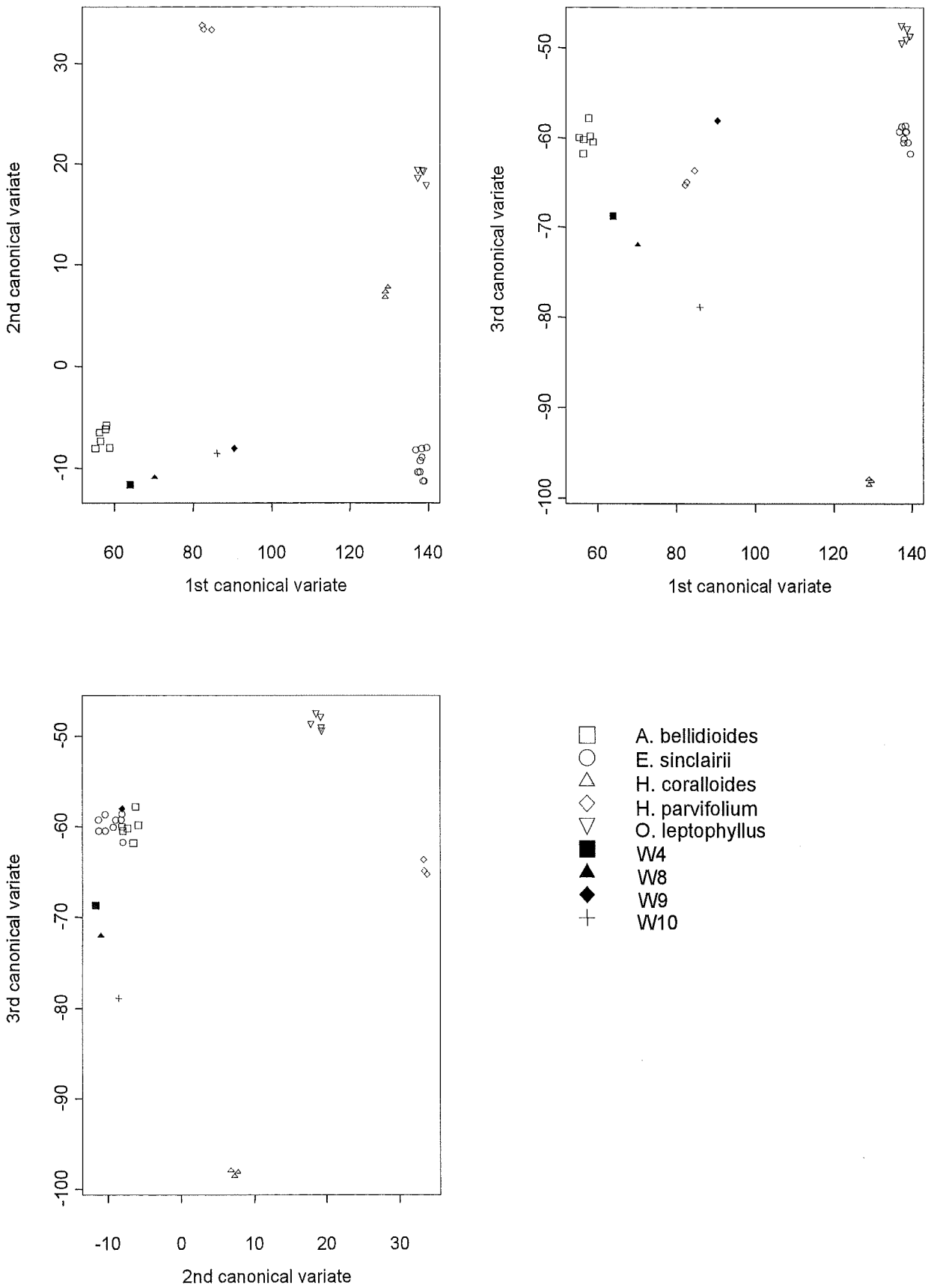


Figure 4.1. Scatter plots of the first, second and third canonical variates for putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, and all sympatric gnaphalioid species. Twenty continuous characters recorded from field-grown specimens were analysed. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

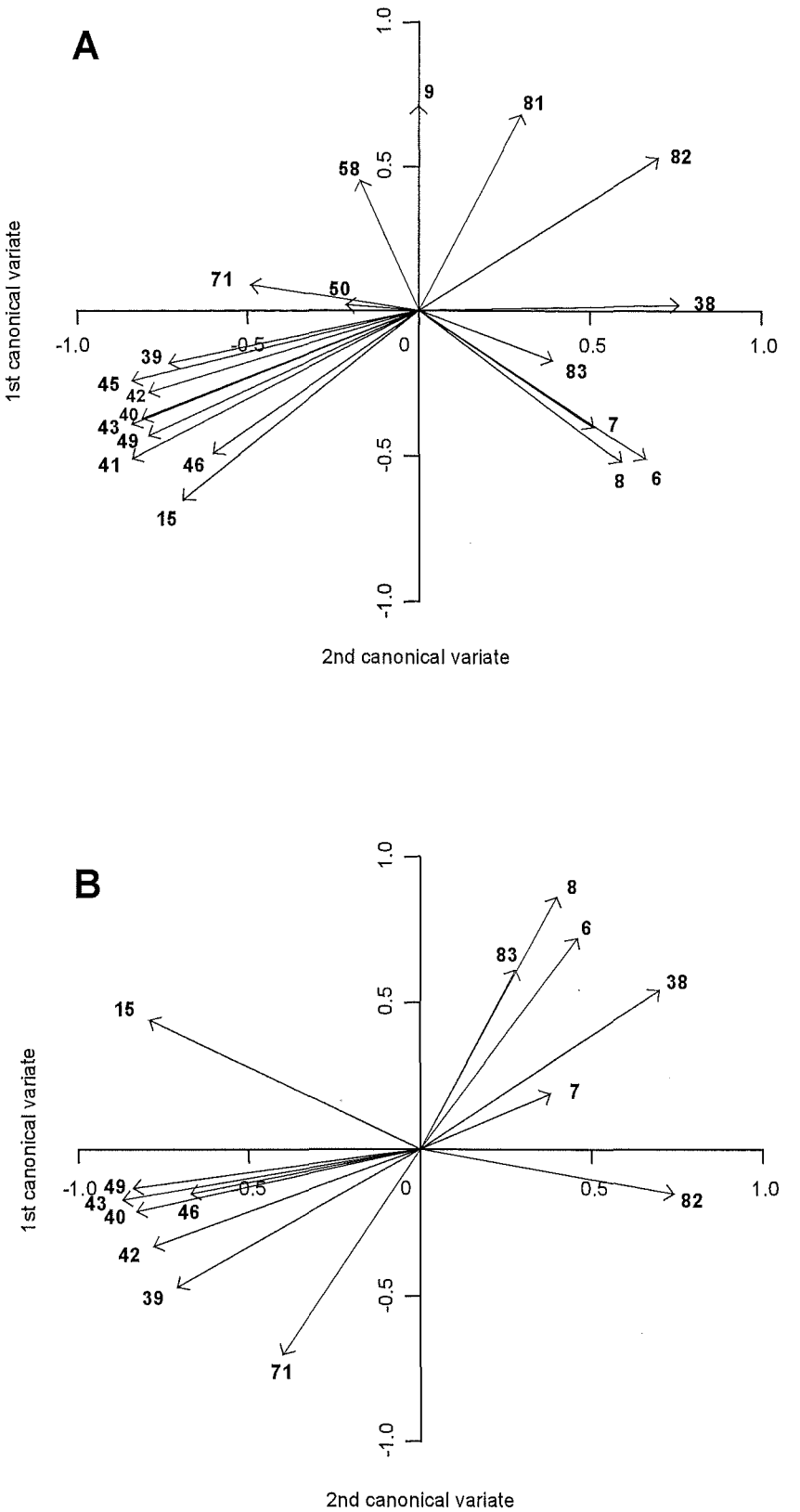


Figure 4.2. Vector plots of Pearson's product-moment correlation coefficients between the original data and the first and second canonical variates following canoncial discriminant analysis of 15 or 20 continuous characters. **A**, 20 continuous characters (see Figure 4.1); **B**, 15 continuous characters (see Figure 4.3).

Character	Canonical variate		
	First	Second	Third
Leaf length	0.6586	-0.5135	0.2921
lamina width	0.5116	-0.3991	-0.1660
Leaf length: maximum lamina width ratio	0.5904	-0.5236	0.4470
Point of maximum lamina width: leaf length ratio	0.0029	0.7069	-0.5365
Mucro length	-0.6949	-0.6494	0.1725
Number of capitula per inflorescence	0.7606	0.0195	0.5766
Capitulum length	-0.7282	-0.1833	-0.5444
Capitulum width at midpoint	-0.8097	-0.3734	-0.3345
Number of female florets per capitulum	-0.8422	-0.5113	-0.2004
Number of hermaphrodite florets per capitulum	-0.7883	-0.2800	-0.4319
Total number of florets per capitulum	-0.8361	-0.3931	-0.3054
Receptacle height	-0.8374	-0.2405	-0.0646
Receptacle diameter	-0.5968	-0.4933	-0.5082
Inner involucral bract length	-0.7938	-0.4296	-0.2609
Inner involucral bract, lamina length	-0.2145	0.0192	-0.1702
Corolla tube length in hermaphrodite florets	-0.1718	0.4451	-0.7409
Pappus hair length in hermaphrodite florets	-0.4857	0.0943	-0.6268
Hermaphrodite floret ovary length	0.2984	0.6778	-0.4452
Hermaphrodite floret ovary width	0.6954	0.5324	0.2462
Hermaphrodite floret ovary length:width ratio	0.3862	-0.1669	0.7048

Table 4.8. Pearson's product-moment correlation coefficients between the original data and the canonical coefficients in the canonical discriminant analysis of 20 continuous characters recorded from field-grown plants (see Figures 4.1 and 4.2 A).

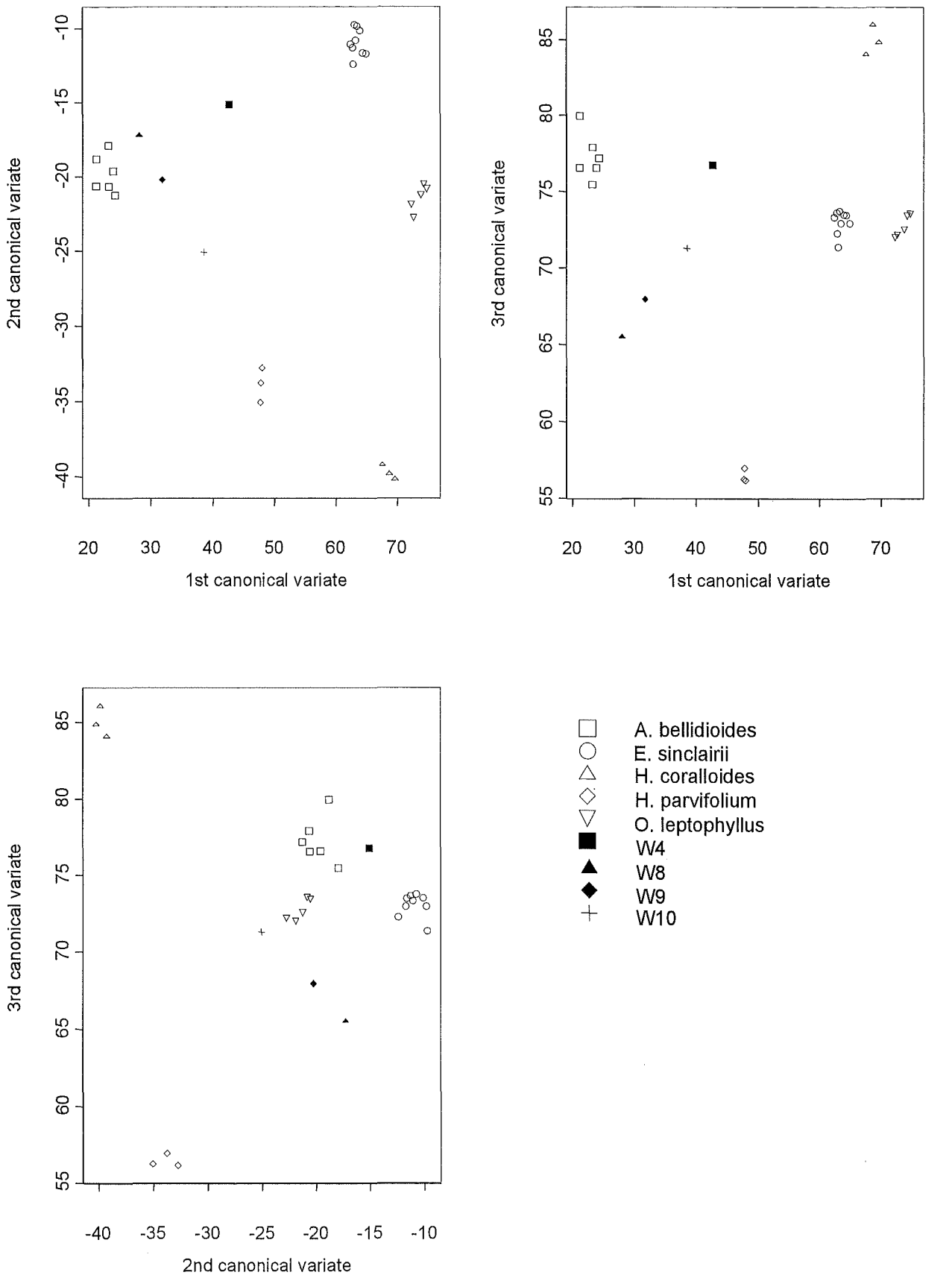


Figure 4.3. Scatter plots of the first, second and third canonical variates for four putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, and all sympatric gnaphalioid species. Fifteen continuous characters recorded from field-grown specimens were analysed. Characters containing outliers were excluded. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

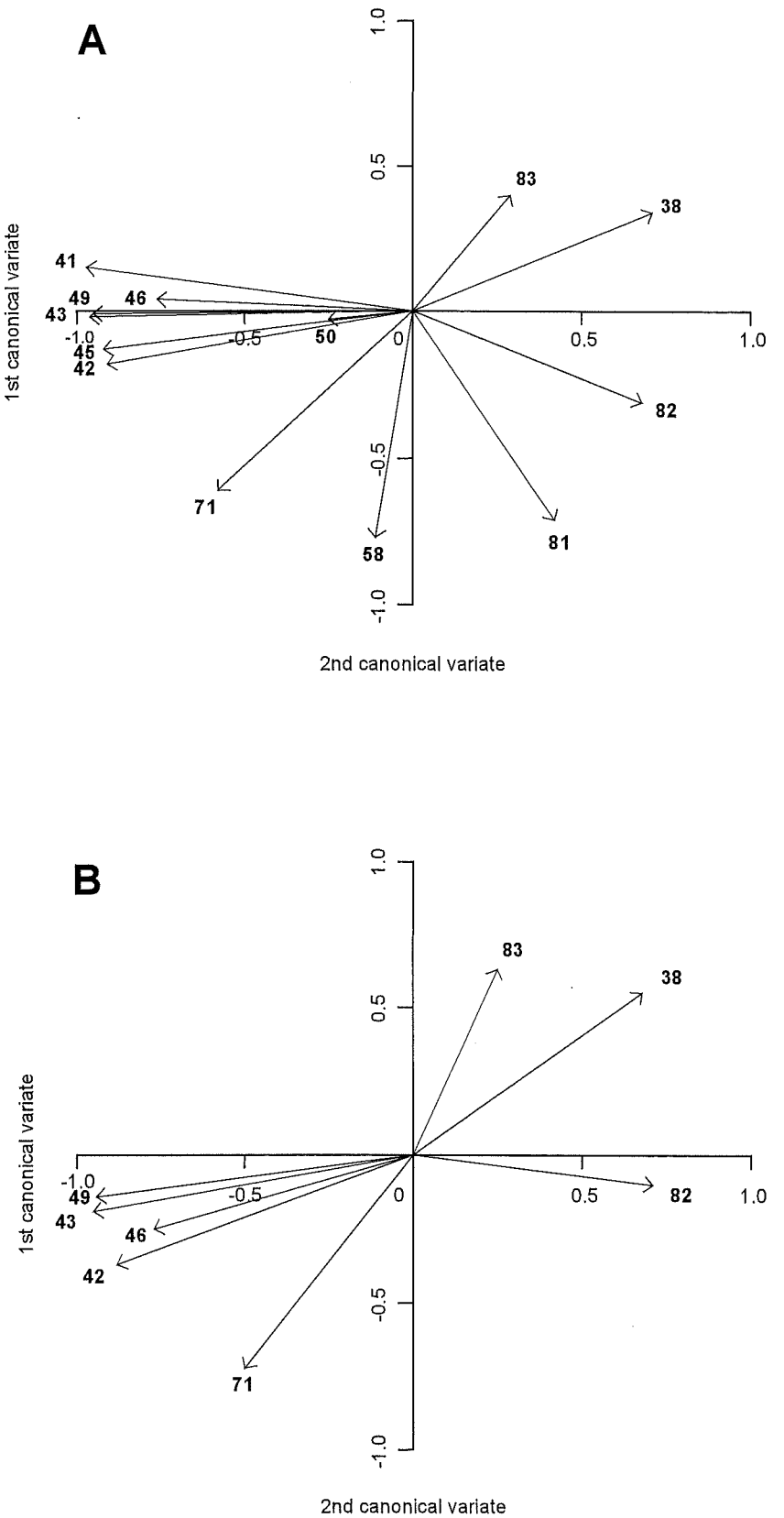


Figure 4.4. Vector plots of Pearson's product-moment correlation coefficients between the original data and the first and second canonical variates following canonical discriminant analysis of eight or 13 continuous characters. **A**, 13 characters; and **B**, eight characters (see Figure 4.5).

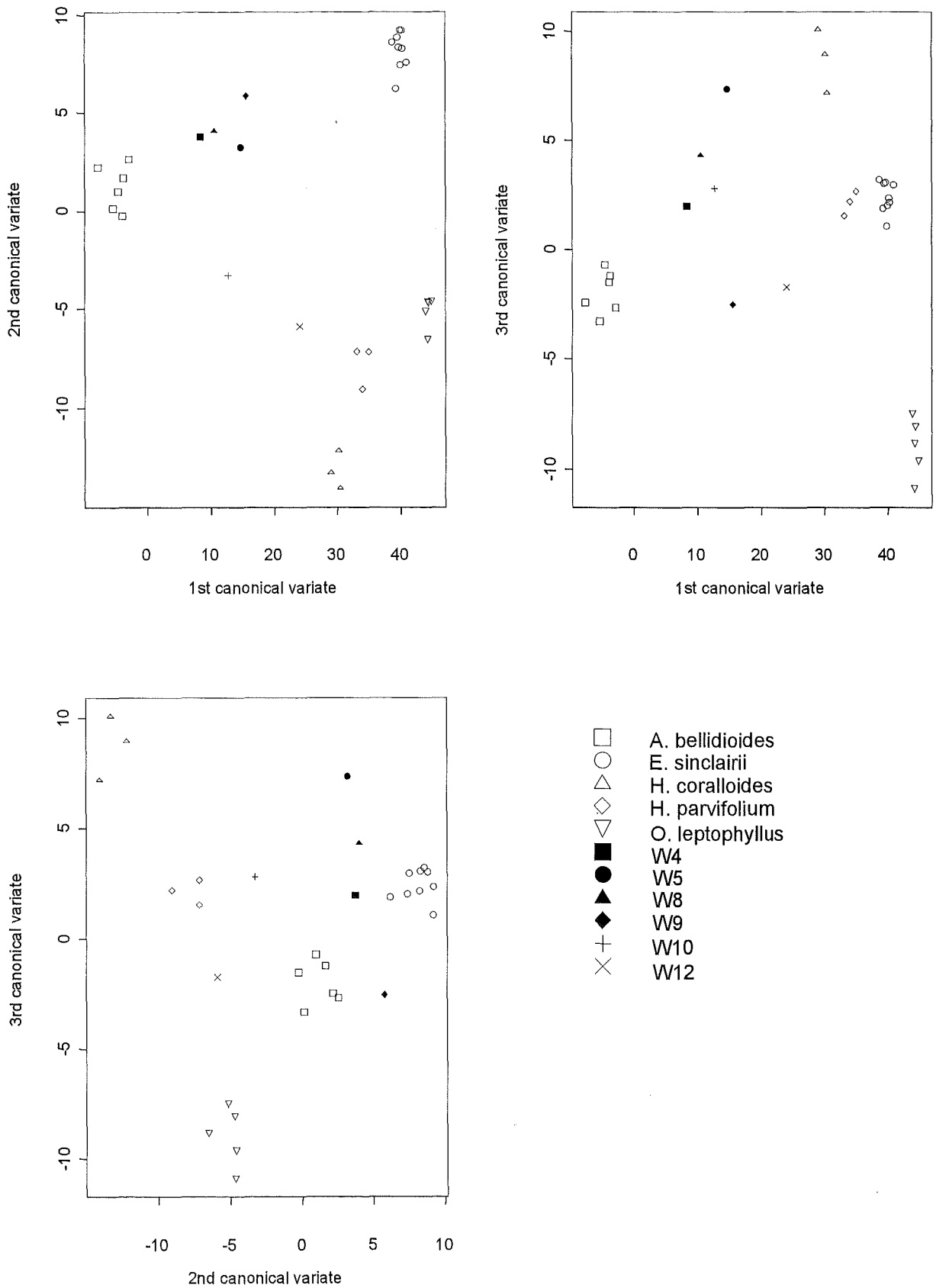


Figure 4.5. Scatter plots of the first, second and third canonical variates for six putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, and all sympatric gnaphalioid species. Thirteen continuous characters recorded from field-grown specimens were analysed. Characters containing outliers were excluded. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

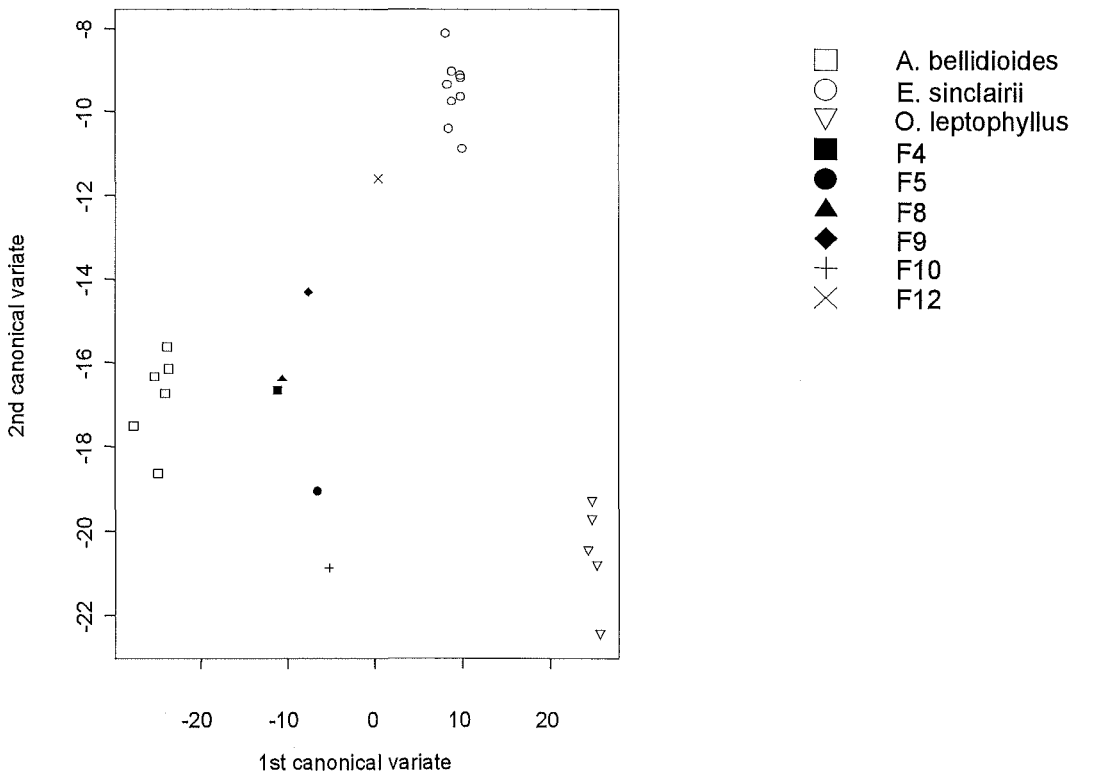


Figure 4.6. Scatter plot of the first and second canonical variates derived from eight continuous characters with *Helichrysum coralloides* and *H. parvifolium* excluded.

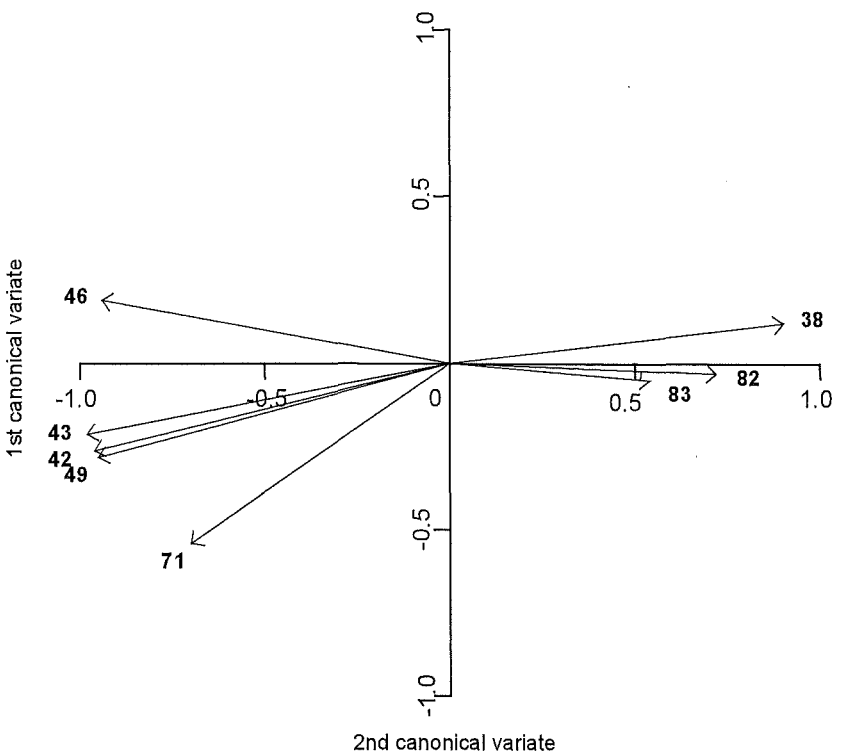


Figure 4.7. Vector plot of Pearson's product-moment correlation coefficients between the original data and the first and second canonical variates following canonical discriminant analysis of eight continuous characters (see Figure 4.6 above).

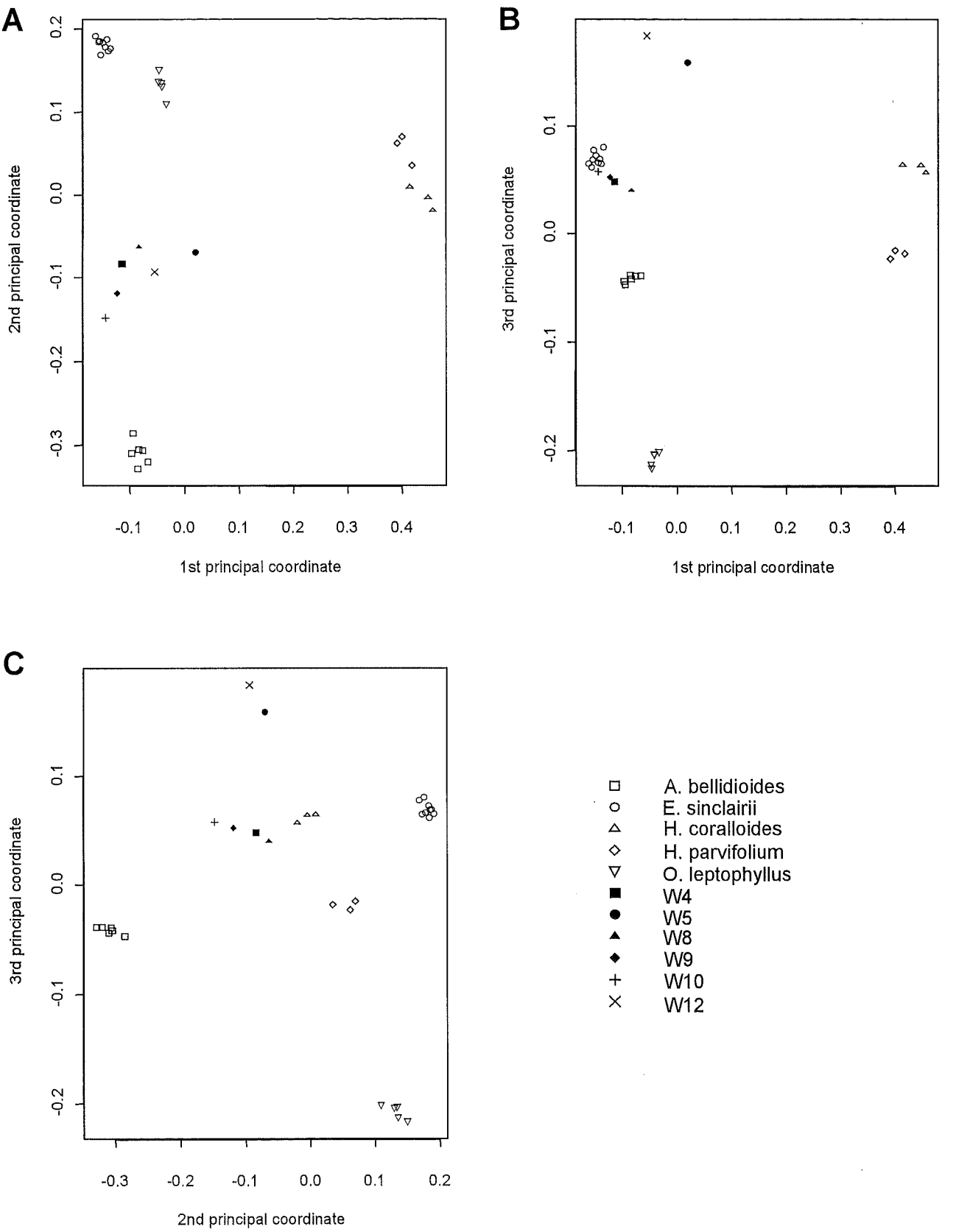


Figure 4.8. Scatter plots of the first, second and third principal coordinates for six putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, and all sympatric gnaphalioid species. Mixed data recorded from field-grown specimens were analysed. **A**, First versus second principal coordinate; **B**, first versus third principal coordinate; **C**, second versus third principal coordinate.

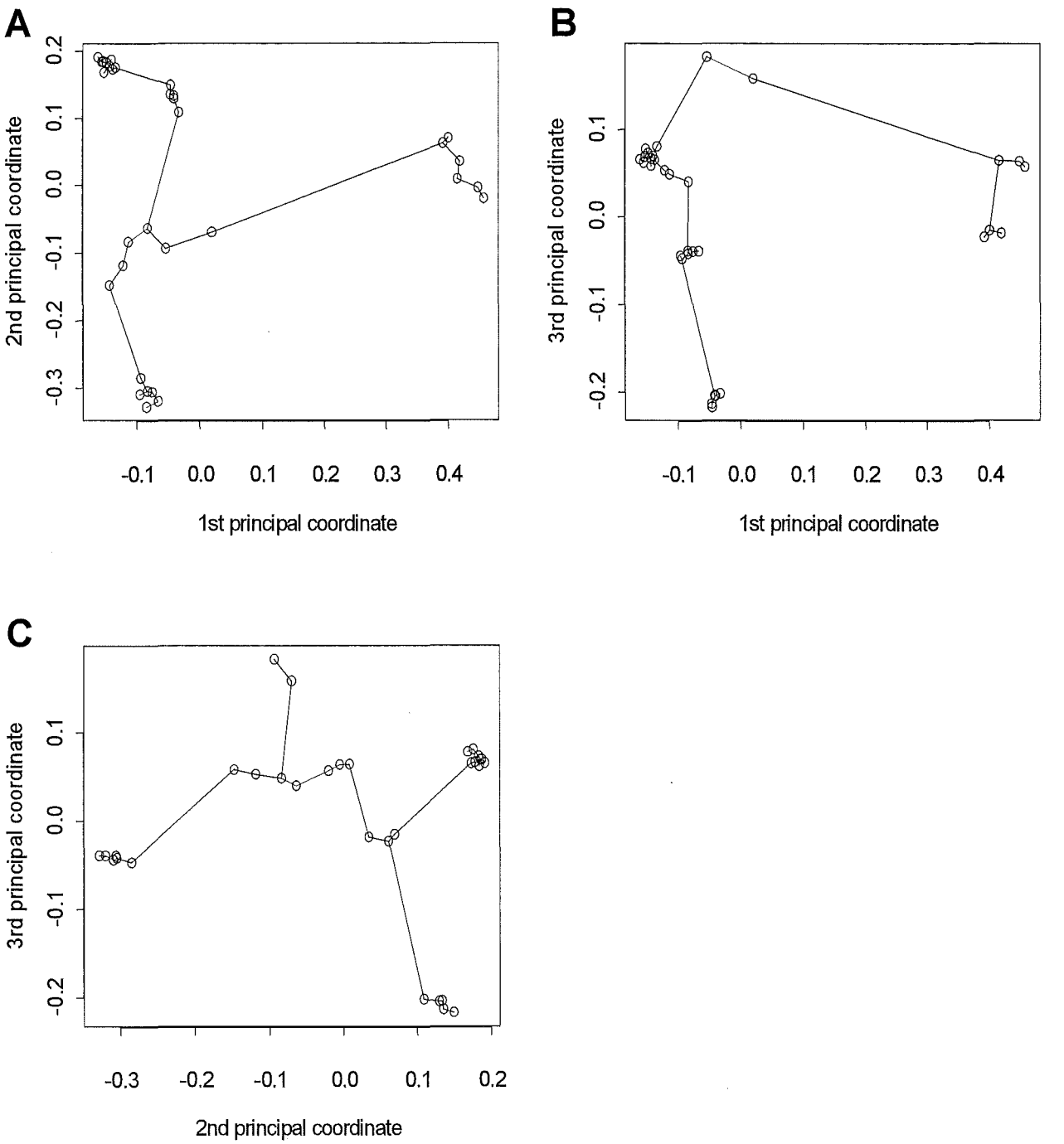


Figure 4.9. Scatter plots of the first, second and third principal coordinates (as presented in Figure 4.8) with a minimal spanning tree constructed on each plot.

Figure 4.10. Scatter plots of the first, second and third principal coordinates for six putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, and all sympatric gnaphalioid species. Continuous and discrete characters recorded from field-grown specimens were analysed separately. **A** and **B**, Analysis of discrete characters; **C** and **D**, analysis of continuous characters.

□ A. bellidioides
○ E. sinclairii
△ H. coralloides
◇ H. parvifolium
▽ O. leptophyllus
■ W4
● W5
▲ W8
◆ W9
+ W10
× W12

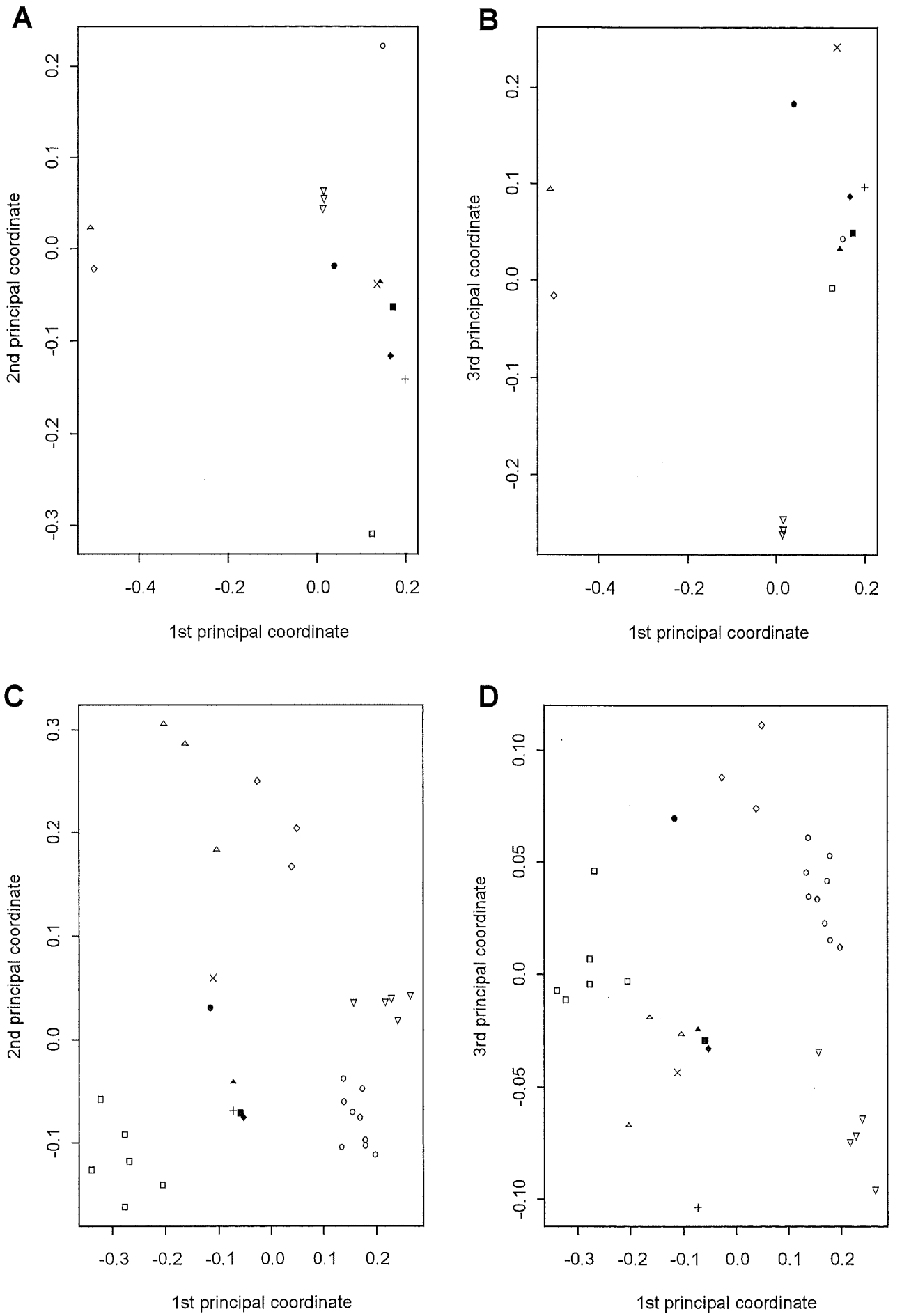

A
2nd principal coordinate
1st principal coordinate
B
3rd principal coordinate
1st principal coordinate
C
2nd principal coordinate
1st principal coordinate
D
3rd principal coordinate
1st principal coordinate

Figure 4.11. Splits graphs generated by split-decomposition analysis of dissimilarities derived from mixed characters for six putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*, and all sympatric gnaphalioid species.

A, All species included (drawn to scale). Fit = 61.6 %.

B, All species included (drawn with equal edges). Fit = 61.6 %.

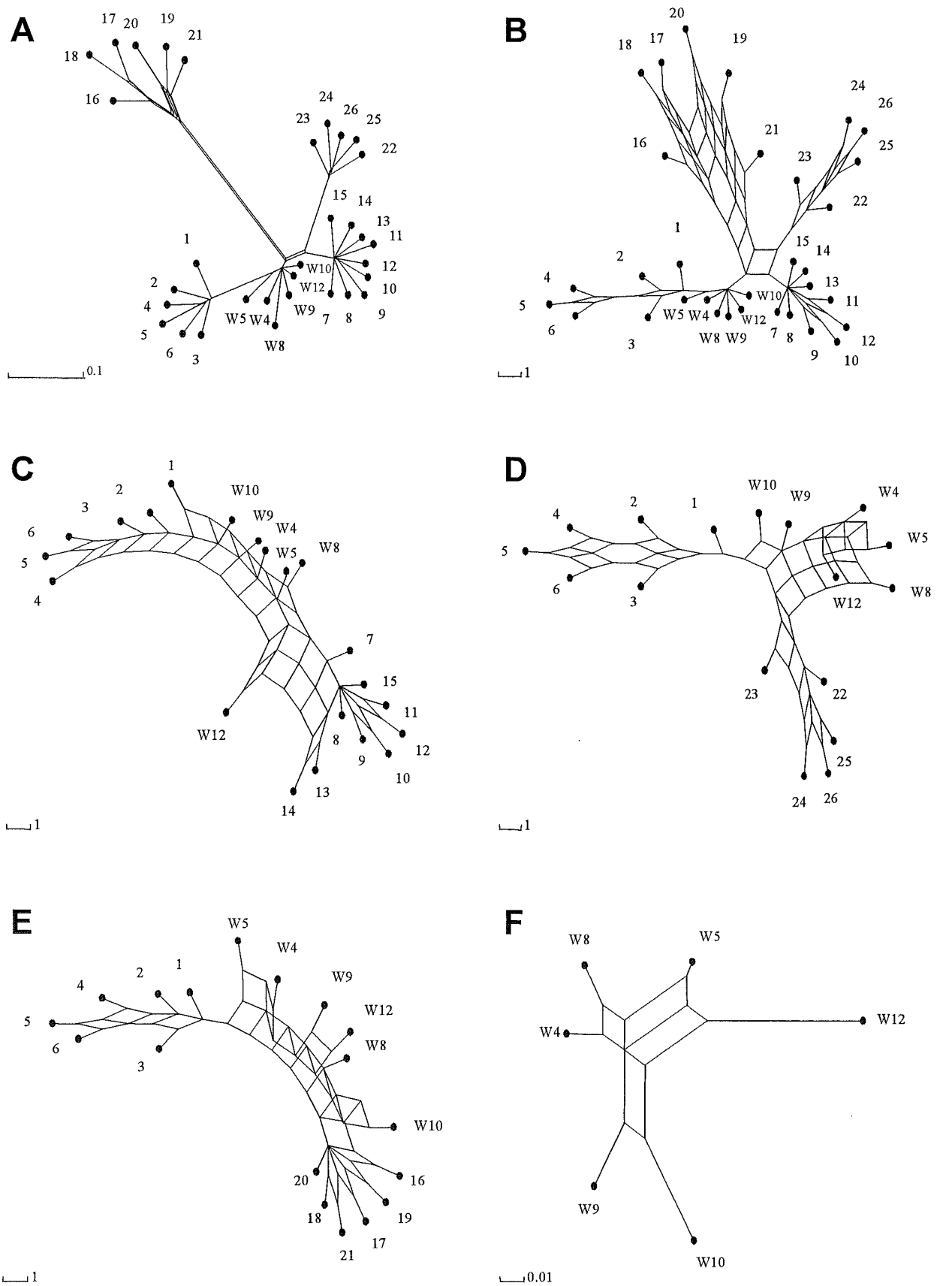
C, *Helichrysum coralloides*, *H. parvifolium* and *Ozothamnus leptophyllus* excluded (drawn with equal edges). Fit = 77.0 %.

D, *Ewartia sinclairii*, *H. coralloides* and *H. parvifolium* excluded (drawn with equal edges). Fit = 90.7 %.

E, *E. sinclairii* and *O. leptophyllus* excluded (drawn with equal edges). Fit = 81.6 %.

F, All species excluded (drawn with equal edges). Fit = 87.0 %.

Key to the species: 1–6, *A. bellidioides*; 7–15, *E. sinclairii*; 16–18, *H. coralloides*; 19–21, *H. parvifolium*; 22–26, *O. leptophyllus*.


A
17 20 19 21
18
16
24 26 25
23
22
15 14
13
11
1
W10
12
2
W12
10
4
W5 W4
W9
7 8 9
5
6 3
W8
0.1
B
20
18 17 19
24
26
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21
25
23
22
1
15 14
2
4
13
W10
11
5
W5 W4
W12
7 8
12
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3
W8 W9
9
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1
C
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2
W10
3
W9
W4
6
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14
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D
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W9
W4
2
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1
W5
5
6
3
W8
W12
23
22
25
24 26
1
E
W5
W4
2
1
4
W9
W12
5
W8
6
3
W10
20
16
18
19
17
21
1
F
W8
W5
W12
W4
W9
W10
0.01

4.3.3.4 Conclusions

The morphology of all of the putative hybrids except *W3* was consistent with a hybrid origin. *W3* was comparable to *Anaphalioides bellidioides* in leaf shape, mucro orientation, leaf indumentum, production of solitary capitula only, pappus-hair morphology, the glabrous ovary of both floret types and the development of crimson pigmentation in the corolla lobes with age. Since only a herbarium voucher of *W3* was available for study, a direct comparison of continuous characters was not possible. *W3* was collected as a possible backcross (J. M. Ward, pers. comm.), but it is concluded to be referable to *A. bellidioides*. The other putative hybrids do not fit into any currently defined species and they do not possess any novel characters that would suggest they belong to an undescribed species. Pollen stainability and the results of experimental crosses provided evidence for reduced fertility in the cultivated putative hybrids. Although reduced fertility is not an absolute indicator of hybridity (Stace, 1975; Stace, 1986), it is a common feature of hybrids, especially between distant related species. Observation of meiotic abnormalities in *W9* and the morphological variability of the putative hybrids, compared to the greater homogeneity of the sympatric gnaphalioid species, are also consistent with a hybrid origin.

Morphological characters strongly suggesting *A. bellidioides* to be one of the parents included leaf lamina shape, the presence of type B glandular trichomes on the leaves, a well-developed mucro, the glabrous ovary of both the female and hermaphrodite florets, capitulum dimensions, floret number per capitulum, and the length of the well-developed, white, hygroscopic lamina on the involucral bracts. Leaf length, the lateral primary nerves raised on the abaxial leaf surface, crimson pigmentation in the anthers, the presence of sparse twin hairs on the ovary of female florets and the glabrous ovary of hermaphrodite florets suggested that *E. sinclairii* was the second parental species. The upturned mucro of *W4*, *W8*, *W9*, *W12*, *W13*, *S1* and *S3* represents a state intermediate between the unique reflexed mucro of *E. sinclairii* and the plane mucro of *A. bellidioides*. Another unique *E. sinclairii* character (crimson pigmentation in the upper corolla tube at anthesis) was not expressed in the putative hybrids.

Ozothamnus leptophyllus was similar to *E. sinclairii* in many characters and so was less readily discriminated. However, no characters unique to *O. leptophyllus* (receptacle scales in the capitula, the absence of female florets and the presence of dense twin hairs on the ovary of hermaphrodite florets) were expressed or suggested in the putative hybrids. Given the strong morphological evidence that *A. bellidioides* was one parent, indumentum density on the adaxial leaf surface also suggested *O. leptophyllus* was not the other parent.

The two whipcord species, *Helichrysum coralloides* and *H. parvifolium*, were morphologically very distinct. Characters unique to these two species included: the presence of a morphologically distinct juvenile phase, tightly appressed adult leaves with involute margins and a cucullate apex, the distribution of clothing trichomes on the leaves, sessile capitula, and twin hairs common on the ovary of *both* the female and hermaphrodite florets. The presence of type B clothing trichomes on the adaxial leaf surface was unique to *H. coralloides*, and acute apical cells on the pappus hairs and twin hairs were unique to *H. parvifolium*. Only in *S1*, in which twin hairs were occasionally observed on the ovary of female and hermaphrodite florets, was any of the above characters suggested in a putative hybrid.

Continuous and discrete characters were inconsistent with regard to identifying the most likely parental species. The contrasting hypotheses suggested by the two character types was highlighted in the HYWIN analyses, in which discrete characters firmly suggested *A. bellidioides* and *E. sinclairii* were the most likely parents, but continuous characters suggested *A. bellidioides* × *O. leptophyllus* was the most likely of a variety of hypotheses. It is likely environmental effects were a major factor in the difference in most continuous characters between field-grown and cultivated clones of *W9* and *W10*, so continuous characters recorded from field-grown specimens were likely to be less reliable than those recorded from glasshouse-cultivated plants. Consequently, greater emphasis was placed on discrete characters to identify the most likely parental species. Continuous floral characters might have had greater predictive value in this study had flowering specimens from cultivated plants of *H. coralloides*, *H. parvifolium* and *O. leptophyllus* been available for study. In addition, the field-grown specimens of putative hybrids were collected in different years (1989 and 1998) and would have been subjected to differing environmental influences. *W12* had only one live shoot when collected; the plant could have been in poor health and would have channelled its available resources into the single shoot, which would probably have influenced continuous characters for this plant.

Canonical discriminant analysis (CDA), metric multidimensional scaling (MDS) and HYWIN provided support for hybridity but varied in the suggested identity of the putative parental species. In scatter plots of the canonical variates and principal coordinates, the putative hybrids formed a separate but heterogeneous group and were consistently placed between *A. bellidioides* and other species. On scatter plots of the first and second canonical variates *W9* was consistently intermediate between *A. bellidioides* and *E. sinclairii*, and *W4*, *W5* and *W8*

were intermediate when leaf and capitulum dimensions were excluded. On scatter plots of principal coordinates *W4*, *W8*, *W9* and *W10* were consistently intermediate between *A. bellidioides* and *E. sinclairii*. However, HYWIN analyses of continuous and mixed data sets suggested *A. bellidioides* × *O. leptophyllus* was the most plausible hypothesis. Only in analyses of discrete characters did HYWIN rank *A. bellidioides* × *E. sinclairii* hypotheses as the most likely, but the program was clearly inefficient when analysing data sets comprising two- or three-state discrete characters only. CDA, HYWIN and split decomposition suggested *W10* and *W12* were the most divergent of the putative hybrids, whereas MDS represented *W5* and *W12* as the most divergent. The putative hybrids were always near the centre of splits graphs and, unlike the species, formed a loose group usually lacking an internal edge. In split-decomposition analyses with all gnaphalioid species included, *H. coralloides*, *H. parvifolium* and *O. leptophyllus* were separated by long internal edges. However, splits graphs generated following sequential exclusion of species from the data set were less conclusive with respect to suggesting hybridity and identifying potential parental species, as the putative hybrids were not always placed predictably.

In conclusion, morphology, cytology and cross-compatibility data were consistent with the suggested hybrid origin for the putative hybrids and that *A. bellidioides* and *E. sinclairii* were the most likely parental species. This hypothesis is tested more fully, utilising cultivated plants, in the following section.

4.3.4 Comparison of the morphology of *Anaphalioides bellidioides*, *Ewartia sinclairii* and the putative hybrids

4.3.4.1 Morphology of *A. bellidioides*, *E. sinclairii* and the putative hybrids

Growth form

Established plants of *A. bellidioides* formed loose mats (Plate 3 A p. 140). The non-flowering shoots were prostrate, they had longer internodes than the flowering shoots, the stems were thicker and at least for the first few nodes the leaves were somewhat smaller. The non-flowering shoots were most conspicuous on seedlings and cuttings and were produced at or below ground level from woody stems. Flowering shoots diverged from the leaf axils at about a 45° angle and thus their initial orientation varied from orthotropic to plagiotropic depending on the position of the bud on the stem, but they ultimately assumed a prostrate habit. The flowering shoots had shorter internodes, thinner stems, and larger leaves. Adventitious root primordia were readily produced at the nodes on all shoots and thus the plant rooted into the substrate as it spread. The orientation of the flowering shoot tips changed to orthotropic with

the development of capitula. The flowering stems bore narrower, bracteate leaves. The capitulum was distinctly pedunculate and the internodes on these shoots continued to elongate until the cypselas were mature. Axillary shoots developed from the leaf axils at the base of the flowering stem and thus enabled vegetative growth to continue.

Plants of *E. sinclairii* had a more upright, subshrubby habit (Plate 3 B p. 140). The orientation of both non-flowering and flowering shoots was ascending to erect. Established plants assumed a spreading, low-growing habit. The non-flowering shoots were less morphologically distinct than in *A. bellidioides* but were most conspicuous in seedlings and cuttings and were produced at or below ground level. Unlike *A. bellidioides* neither shoot type produced adventitious root primordia towards the apex and adventitious roots developed at the base of woody shoots only. The internodes of the flowering stems were longer and the leaves narrower and shorter. From three to 64 capitula were borne in terminal cymose inflorescences on each flowering shoot. The capitulum peduncles elongated prior to and after anthesis so that the capitula were distinctly pedunculate when the cypselas were shed.

The cultivated putative hybrids were very similar in growth form (Plate 3 C–F p. 140). They were not as obligately prostrate as *A. bellidioides*, but the vegetative shoots were incapable of maintaining an erect orientation and are described as decumbent. Established plants formed loose mats but with more ascending shoots than in *A. bellidioides*. The vegetative shoots were morphologically distinguishable and similar to those of *A. bellidioides*. Orientation of the flowering shoots was initially variable, gradually assuming a prostrate orientation but becoming orthotropic with the development of capitula. Adventitious roots developed from the nodes of vegetative shoots but not as precociously as in *A. bellidioides*. The habit of *S1* was similar to that of *A. bellidioides* and more prostrate than the field-collected putative hybrids, whereas the habit of *S2* and *S3* was more similar to the field-collected putative hybrids.

The plant of *W12* growing at the study site had a sprawling habit and the sole flowering shoot was erect. Data on the growth habits of putative hybrids collected by Ward and Lovis (*W1–W6*) was unavailable, but photographs of these plants showed they were prostrate or sprawling with erect, morphologically distinct flowering shoots.

As previously mentioned (p. 99), seedlings of *A. bellidioides* and *E. sinclairii* lacked a morphologically distinct juvenile phase. The seed-raised putative hybrids also lacked a

distinct juvenile form and new growth on cuttings from field-collected putative hybrids did not revert to a morphologically distinct leaf form.

Leaf morphology

In *A. bellidioides* the lamina was narrowly obovate and narrowed noticeably towards the petiole (Figure 4.12 A p. 144). The lamina was oblanceolate in *E. sinclairii* and tapered evenly towards the petiole (Figure 4.12 B). In the putative hybrids the leaves were narrowly obovate to obovate and the lamina was tapering (as in *W8*) or narrowed towards the petiole (Figure 4.12 C–K). In both species and all putative hybrids, the leaf tip and lamina margins were plane. The leaf tip was obtuse in *A. bellidioides*, rounded in *E. sinclairii* and varied among the putative hybrids from obtuse to rounded.

Leaf dimensions varied considerably among the cultivated putative hybrids, but virtually all leaves measured fell within the range of variation of *A. bellidioides* and *E. sinclairii* individuals (Figure 4.13 p. 145). Some leaves of *W1* and *W10* were slightly narrower than those of *A. bellidioides* but the means were similar. The leaves of *W2*, *W11* and *W13* were of similar dimensions to *E. sinclairii* leaves, whereas leaf dimensions in *W1*, *W10* and *S1* were more similar to *A. bellidioides*. The leaves of *W9* were intermediate in size between the two species. The leaves of *W12* were slightly smaller than field-collected leaves of both species but intermediate with respect to the length:width ratio. The leaves of *W4* and *W8* were similar in size to field-collected leaves of *A. bellidioides*. Leaves were absent from the preserved shoots of *W5*.

In *A. bellidioides* the petiole extensions enclosed 75–90 % of the stem and ran either the full length of the internode or almost so. In *E. sinclairii* the petiole extensions ended midway down the internode and enclosed 50 % of the stem. In the putative hybrids the petiole extensions extended 50–90 % of the length of the internode and enclosed 50–75% of the stem.

The leaves of both species and all putative hybrids were mucronate. The mucro was 350–450 μm long in *A. bellidioides* and was plane with the leaf axis. The mucro in *E. sinclairii* was 150–200 μm long and was reflexed, pointing towards the leaf axil. It was always concealed by the leaf indumentum. The mucro ranged from 275–475 μm long in the putative hybrids (Table 4.6 p. 109). The mucro usually pointed upwards (at up to a 45° angle) in *W4*, *W8*, *W9*,

W13, *S1* and *S3* but was never concealed by the leaf indumentum. In the other putative hybrids the mucro was plane with the lamina.

In both species and all putative hybrids, the uniseriate clothing trichomes formed a dense, felt-like indumentum on the abaxial leaf surface. The density of the clothing trichomes on the adaxial leaf surface was sparse to glabrous in *A. bellidioides* and dense in *E. sinclairii*. The density on the adaxial surface was moderate in all field-collected putative hybrids and sparse in *S1*, *S2* and *S3*. In *A. bellidioides* the clothing trichomes had only one basal cell and the base of the terminal cell was not swollen. In *E. sinclairii* the clothing trichomes had one or two basal cells and the terminal cell had a distinctly swollen base. In the putative hybrids the clothing trichomes had always one, or one to two, basal cells and the base of the terminal cell was often, but not always, swollen.

Type A glandular trichomes were present on both leaf surfaces in both species and in the putative hybrids (Figure 4.14 A–C p. 145). These trichomes were always sparse on the adaxial surface and more common on the abaxial surface, particularly towards the leaf base. Only minor variation in the distribution, structure and length of these trichomes was observed among the two species and the putative hybrids. The trichome length was similar in *E. sinclairii* (50–80 μm long) and *A. bellidioides* (50–90 μm long). The terminal cells were oval in shape and 12–16 μm long in *A. bellidioides*, but oblong and 28–35 μm long in *E. sinclairii*. In the putative hybrids the terminal cells were oblong-oval in shape and 15–20 μm long.

Type B glandular trichomes were common on the adaxial surface and margins of the leaves in *A. bellidioides* (Figure 4.14 D) but were not observed in *E. sinclairii*. They were sparse to moderate in density in *A. bellidioides* and were 75–150 μm long and 25–70 μm wide at the base. Type B trichomes were also observed in the putative hybrids (Figure 4.14 E) but were uncommon, smaller (e.g., 60–90 μm long and up to 30 μm wide in *W1*) and there was often little distinction from type A glandular trichomes.

Leaf anatomy

In both *A. bellidioides* and the putative hybrids, the cuticle and epidermis was thicker on the adaxial surface, whereas in *E. sinclairii* they were of equal thickness on the adaxial and abaxial surfaces. In *A. bellidioides* the guard cells were level with the epidermis, but in *E. sinclairii* the guard cells and adjacent epidermal cells were raised. The guard cells were level

with the epidermis in *S2* and *S3*, but in the other putative hybrids the guard cells were raised above the epidermis.

The leaves of both species and the putative hybrids had a dorsiventral lamina structure with one or two layers of palisade chlorenchyma cells below the adaxial epidermis. The thickness of the chlorenchyma layers was similar in all individuals. However, the degree of differentiation of the spongy chlorenchyma differed. In *A. bellidioides* the spongy chlorenchyma was well differentiated, the intercellular spaces were large, and the central cells were large and elongate (occasionally over 150 μm in diameter in transverse section). In *E. sinclairii* the central cells of the spongy chlorenchyma were smaller (up to 60 μm in diameter but usually less) and more spherical in shape, and the intercellular spaces were smaller. Palisade and spongy chlorenchyma layers were present but less distinct than in *A. bellidioides*. In the putative hybrids the central cells of the spongy chlorenchyma were oblong to rounded in shape and intermediate in size between those of the two species (usually about 40–65 μm in diameter). Distinct palisade and spongy chlorenchyma layers were present in all putative hybrids, with differentiation least distinct in *S1*. The palisade chlorenchyma was continuous across the midrib in *A. bellidioides* and all of the putative hybrids, but not in *E. sinclairii*.

Collenchyma was present on the abaxial side of the vascular bundle in the midrib and largest lateral ribs in *E. sinclairii*, but was absent in *A. bellidioides*. Some collenchyma cells were present on the abaxial side of the midrib in the putative hybrids.

Leaf venation

The leaf venation of *A. bellidioides*, *E. sinclairii* and the putative hybrids was very similar, all having trinervate leaves with a reticulate venation pattern (Figure 4.12 A–K p. 144). Higher-order nerve branching occurred closer to the leaf base in *E. sinclairii* than in *A. bellidioides*, and the lateral primary nerves were longer relative to the overall leaf length in *E. sinclairii*. The leaf venation of the putative hybrids was extremely similar to *A. bellidioides* and *E. sinclairii* with relatively little variation between individuals. The midrib and lateral nerves were prominently raised on the abaxial leaf surface in *E. sinclairii*. They also protruded in the putative hybrids but to a lesser degree. In *A. bellidioides* only the midrib was visibly raised on the abaxial leaf surface.

Floral morphology

Cultivated plants of both putative parental species and all putative hybrids had similar flowering periods, with flowering occurring between late September and late November in the glasshouse.

In *A. bellidioides* a solitary, terminal capitulum was carried on each flowering shoot (Plate 4 A p. 141), whereas in *E. sinclairii* 3-64 capitula were borne in dense terminal cymose inflorescences on each flowering shoot (Plate 4 B). In all field-collected putative hybrids, the capitula were either solitary or borne in terminal cymose inflorescences (Plate 4 C & D). For plants in which more than one flowering shoot was available for study, the number of capitula per inflorescence varied. The largest inflorescences observed contained eight capitula in *W9* and *W13*. Both solitary capitula and multicapitulate inflorescences containing up to seven capitula were produced by *S1*, but the capitula were always solitary in *S2* and *S3*.

The capitula of *A. bellidioides* were considerably larger and more variable in size than in *E. sinclairii* (Figure 4.15 p. 146). The capitula of all of the putative hybrids were intermediate in size between those of *A. bellidioides* and *E. sinclairii*. The mean capitulum width ranged from 3.4 mm in *W9* to 4.7 mm in *W2* and *W5*, and the mean capitulum length ranged from 5.7 mm in *W3* to 7.1 mm in *W10*.

The receptacle in all *A. bellidioides* individuals was conical and of the scrobiculate type (Plate 5 A p. 142). The receptacle was convex in three *E. sinclairii* individuals and plane in two individuals. The receptacle surface of some cultivated *E. sinclairii* plants was fimbrillate, with green rudimentary scales up to 475 μm long (but usually about 200 μm long) encircling the base of each floret (Plate 5 B). In two *E. sinclairii* individuals the receptacle was foveolate with only small ridges or projections around the floret bases. In all putative hybrids the receptacle was subconical (Plate 5 C & D). Small projections or ridges were present around the floret bases in the field-collected putative hybrids but were smaller than those found in *E. sinclairii*. The seed-raised putative hybrids had a scrobiculate receptacle. All of the putative hybrids were intermediate between *A. bellidioides* and *E. sinclairii* with respect to receptacle height and diameter (Figure 4.16 p. 146), but in *S2* the receptacle dimensions were more similar to *A. bellidioides*.

In both species and all putative hybrids, the inner involucral bracts had a white hygroscopic lamina. The outer bracts, in particular, had dense indumentum on the abaxial surface of the

stereome. In the outermost involucral bracts the lamina-stereome gap was reddish-purple in *E. sinclairii* and pale brown in *A. bellidioides*. The gap of the outermost bracts was often flushed with red in *W1*, *W11*, *W12* and *W13*, but was pale brown in the other putative hybrids for which data was available. The tip of the inner bracts was rounded in *E. sinclairii* and acute in *A. bellidioides* (Figure 4.17 A & B p. 147). In the putative hybrids the tips of the inner bracts were either acute (*S2*), acute to obtuse (*W10*, *W12* and *S1*), or obtuse to rounded in the other putative hybrids (Figure 4.17 C–F). The stereome had distinct hyaline margins in *E. sinclairii* but these were absent in *A. bellidioides*. Narrow hyaline margins were present on the stereome in all putative hybrids except *S1*. Both the lamina length and total bract length were considerably higher in *A. bellidioides* than in *E. sinclairii* (Figure 4.18 p. 148). In these two characters all of the putative hybrids were intermediate. The bracts were longest in *W2* and *S1*, and shortest in *W1* and *W11*.

The capitula contained female and hermaphrodite florets in both species and all putative hybrids. *A. bellidioides* and *E. sinclairii* were clearly differentiated by the female: hermaphrodite floret ratio and floret number per capitulum (Figure 4.19 p. 148). In *E. sinclairii* the capitula contained 26–46 florets, of which 24–38 % were female florets. In *A. bellidioides* the capitula contained 191–298 florets, of which female florets comprised 50–57 %. All putative hybrids were intermediate in the numbers of female and hermaphrodite florets and total floret number per capitulum. However, *S2* and *S3* were closer to *A. bellidioides* in these characters. The sex ratios of the putative hybrids were also intermediate with the exception of *S3*, which produced 58–62 % female florets, and in *W4* and *W8* the sex ratio was similar to *A. bellidioides*. Total floret number in the single capitulum of *W12* was approximately half that of the cultivated putative hybrids, but the sex ratio (44 % female florets) was within the range of variation of the cultivated putative hybrids.

The corolla lobes of the hermaphrodite florets were erect in *A. bellidioides* and recurved in *E. sinclairii* at anthesis (Figure 4.20 p. 149). At least some of the corolla lobes were patent or recurved in all of the putative hybrids except *S1*, in which the corolla lobes were consistently erect. In all plants studied the corolla colour (and changes in colour) were identical in both the female and hermaphrodite florets. The corolla lobes were pale green in *A. bellidioides* and the seed-raised putative hybrids, white in *E. sinclairii* and *W12*, and greenish-white in the other putative hybrids for which data was available. The lower half of the corolla tube was pale green in both species and the putative hybrids. At anthesis the upper corolla tube was crimson in *E. sinclairii*, and pale green in *A. bellidioides* and all cultivated putative hybrids, but it

became flushed reddish-purple with age in some *A. bellidioides* individuals, *W1*, *W10* and *S3*. The upper corolla tube was crimson in the partially fruiting capitulum of *W12*.

The corolla tube of both the female and hermaphrodite florets was markedly longer in *A. bellidioides* than in *E. sinclairii* (Figure 4.21 p. 150). Surprisingly, individuals of *A. bellidioides* formed two distinct groups. All of the putative hybrids were intermediate between *A. bellidioides* and *E. sinclairii* with regard to corolla tube length, but there was some variation between individuals. *W4*, *W5*, *W8*, *W9* and *W11* were more similar to *A. bellidioides*, whereas *S2* was most similar to *E. sinclairii*. In *W12* the corolla tube of both floret types was slightly longer than those of field-collected specimens of *A. bellidioides*.

The ovary of both floret types was glabrous in *A. bellidioides* (Plate 6 A p. 143). Occasional twin hairs were present on the ovary of female florets in two *E. sinclairii* individuals (Plate 6 B), but none were observed on the hermaphrodite florets. Sparse twin hairs were present on the ovary of female florets in *W1*, *W2*, *W5*, *W10* (Plate 6 C & D), *W12* and *W13*, and a single twin hair was observed on the ovary of a female floret in *S2*. *S1* was unique in that twin hairs were occasionally observed on the ovary of hermaphrodite florets and a single multicellular twin hair (90 μm long) was observed on the ovary of a female floret. The twin hairs were 35–45 μm long in *E. sinclairii* and 70–500 μm long in the putative hybrids. The ovary epidermal cells were rounded in *E. sinclairii*, flat in *A. bellidioides*, and flat to slightly rounded in the putative hybrids.

With respect to pappus hair length, the putative hybrids were either intermediate between the putative parental species or similar to *A. bellidioides* (Figure 4.22 p. 150). Most of the putative hybrids fell within the range of variation of *A. bellidioides*. Only *W1*, *W11* and *S2* were intermediate between the putative parental species, but all three putative hybrids were closer to *A. bellidioides* than *E. sinclairii*. The pappus apical cells were rounded in both species and all of the putative hybrids (Figure 4.23 A–H p. 151). In *E. sinclairii* the apical cells had distinctly reticulate wall thickening, but in *A. bellidioides* they had uniformly thickened walls. In all of the putative hybrids the apical cells had reticulate or irregularly thickened walls with a sculptured appearance when viewed with brightfield optics. In *E. sinclairii* the pappus hairs of the female florets were dimorphic, the two forms bearing 1–2 non-protruding or 3–5 protruding apical cells; the pappus hairs of the hermaphrodite florets had 4–6 protruding apical cells and were slightly broader at the tip. In *A. bellidioides* the pappus hairs were monomorphic and had 1–2 non-protruding apical cells. In all putative

hybrids the pappus hairs of the female and hermaphrodite florets differed. In most field-collected putative hybrids the pappus hairs were monomorphic on each floret with 2–4 slightly protruding apical cells in the female florets and 2–5 protruding apical cells in the hermaphrodite florets. However, *W9* and *W12* differed in having dimorphic pappus hairs with 2–3 non-protruding apical cells in the female florets and 3–5 protruding apical cells in the hermaphrodite florets. In the seed-raised putative hybrids, the apical cells did not protrude in either floret types and varied in number from 2–4 in the female florets to 2–5 in the hermaphrodite florets. The basal cilia were sparse, very short (3–12 μm) and ascending in *A. bellidioides*, and longer (5–30 μm) and ascending to spreading in *E. sinclairii* (Figure 4.23 I–O). No difference in the basal cilia of female and hermaphrodite florets were noted in either species. The basal cilia were intermediate in density and length (e.g., 5–18 μm in *W1*) and ascending to spreading in the putative hybrids.

Crimson pigmentation was present in the anthers in *E. sinclairii* but was absent in *A. bellidioides*. The anthers of the field-collected putative hybrids (for which data was available) had a pale reddish coloration, but the seed-raised putative hybrids lacked any pigmentation in the anthers. The pollen was yellow in *A. bellidioides* and white in *E. sinclairii*. All putative hybrids produced yellow or pale yellow pollen except for *W13*, in which the pollen was white or cream.

Plate 3. Growth form of *Anaphalioides bellidioides*, *Ewartia sinclairii* and putative hybrids between the two species.

A, *A. bellidioides* (photo Dougal Holmes).

B, *E. sinclairii* growing on the Yeo Stream bank (photo John Lovis).

C, *W10* (photo Dougal Holmes).

D, *W9* and *A. bellidioides* growing at the study site.

E, *S1* (photo Dougal Holmes).

F, *S2* (photo Dougal Holmes).

Scale = 5 cm.

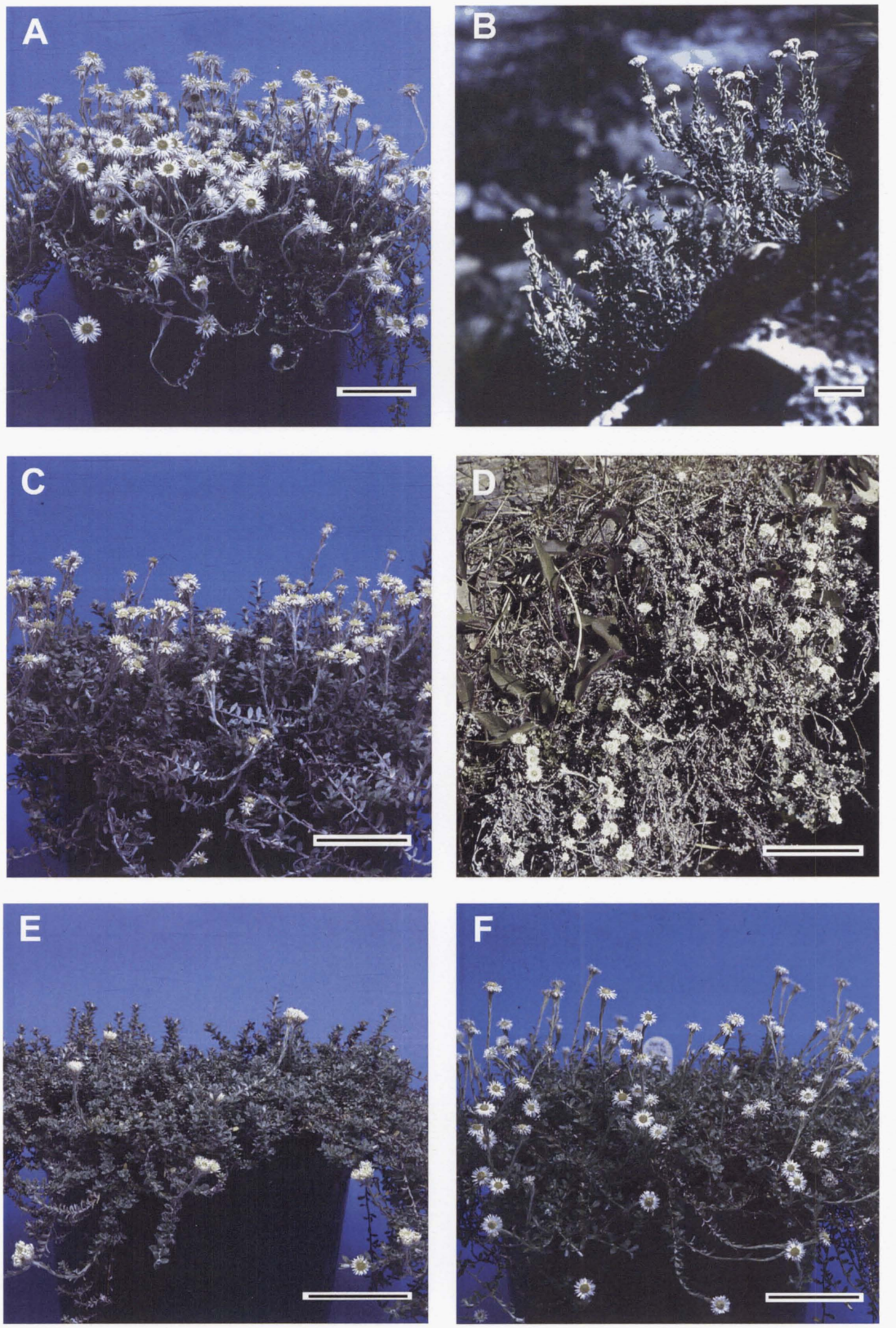

A
B
C
D
E
F

Plate 4. Capitula of *Anaphalioides bellidioides*, *Ewartia sinclairii* and putative hybrids between the two species.

A, *A. bellidioides.* Scale = 1 cm (photo Dougal Holmes).

B, *E. sinclairii.* Scale = 50 mm (photo Dougal Holmes).

C, *W10.* Scale = 25 mm.

D, *S2.* Scale = 50 mm (photo Dougal Holmes).

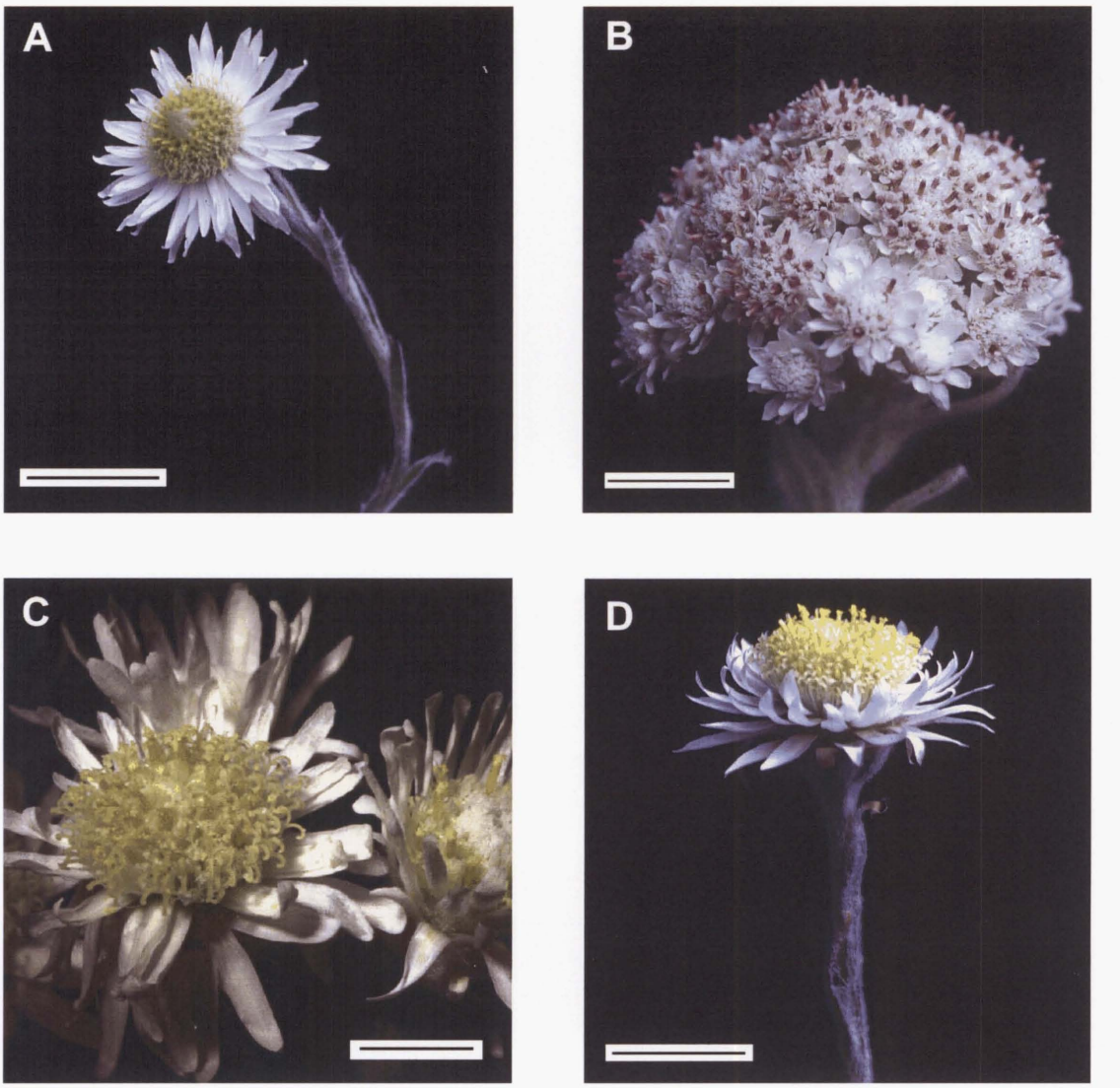

A
B
C
D

Plate 5. Receptacle of *Anaphalioides bellidioides*, *Ewartia sinclairii* and putative hybrids between the two species.

A, *A. bellidioides* (photo Neil Andrews).

B, *E. sinclairii* (photo Neil Andrews).

C, *W9* (photo Neil Andrews).

D, *W13* (photo Neil Andrews).

Scale = 300 μm.

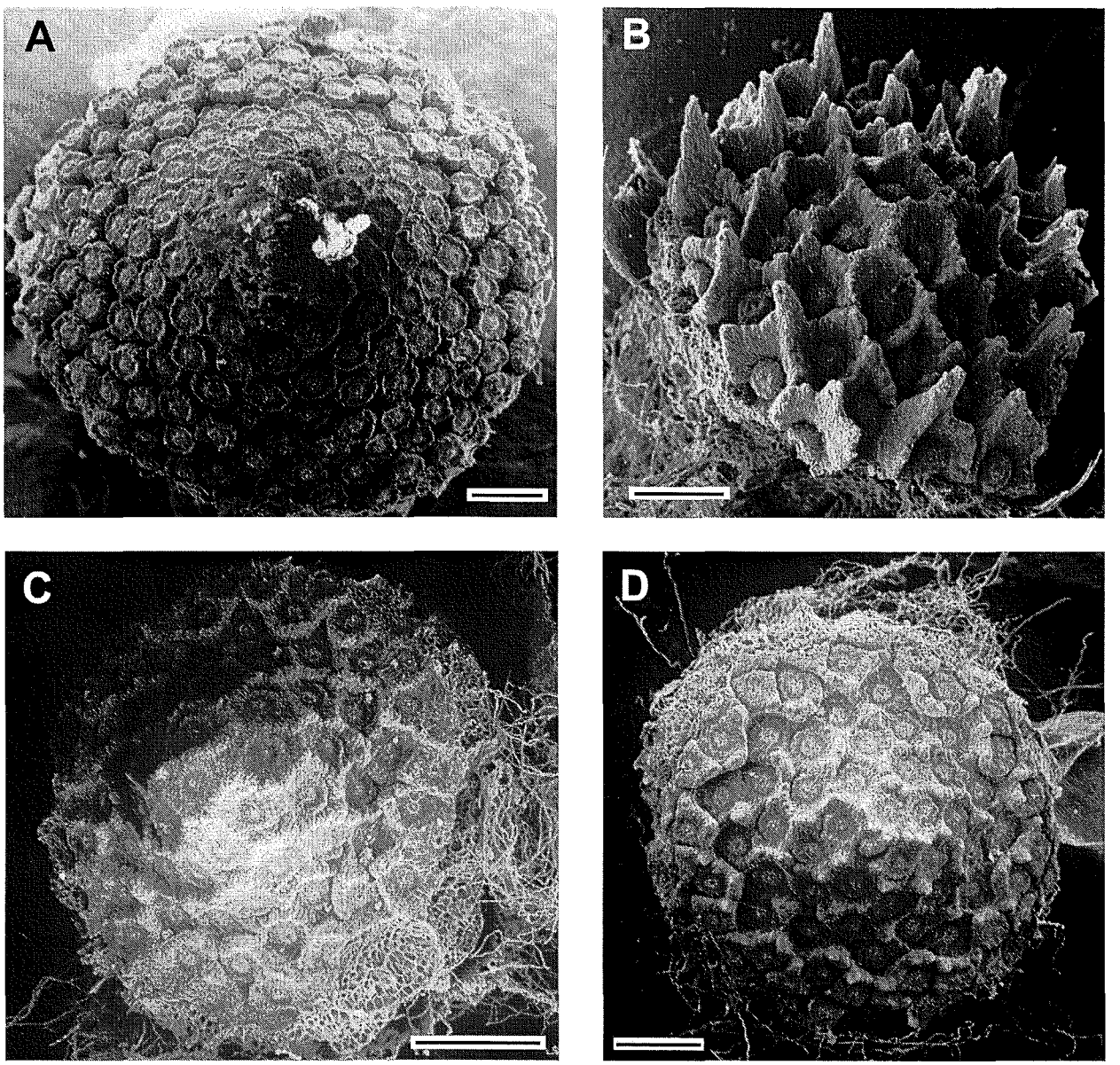

A
B
C
D

Plate 6. Ovary of female florets of *Anaphalioides bellidioides*, *Ewartia sinclairii* and putative hybrids between the two species.

A, *A. bellidioides*. Scale = 100 μm (photo Neil Andrews).

B, *E. sinclairii*. Scale = 200 μm (photo Neil Andrews).

C, *W10*. Scale = 100 μm (photo Neil Andrews).

D, a twin hair on the ovary in *W10*. Scale = 10 μm (photo Neil Andrews).

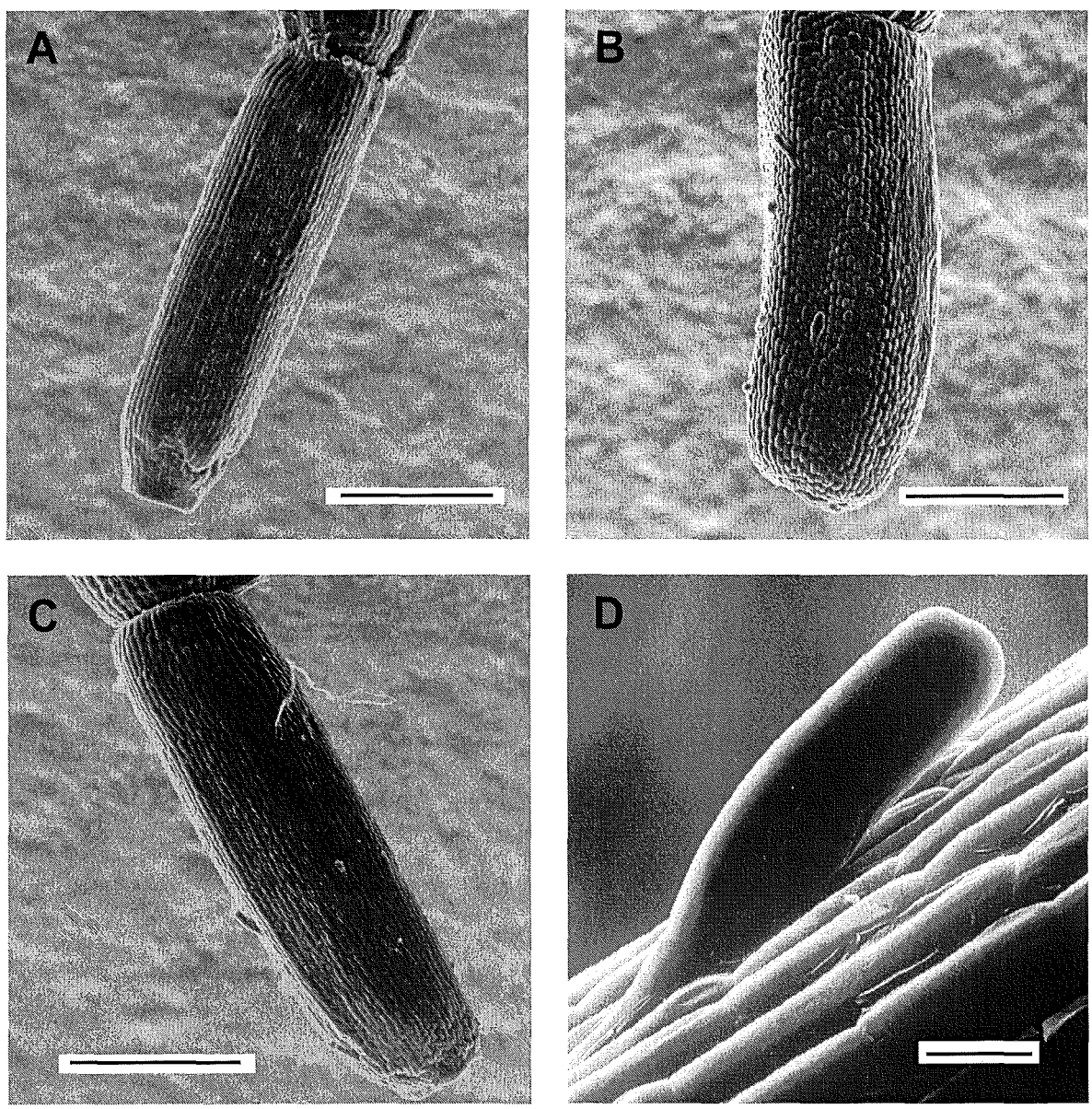

A
B
C
D

Figure 4.12. Leaf shape and venation of *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

A, *A. bellidioides.*

B, *E. sinclairii.*

C, *W1.*

D, *W2.*

E, *W9.*

F, *W10.*

G, *W11.*

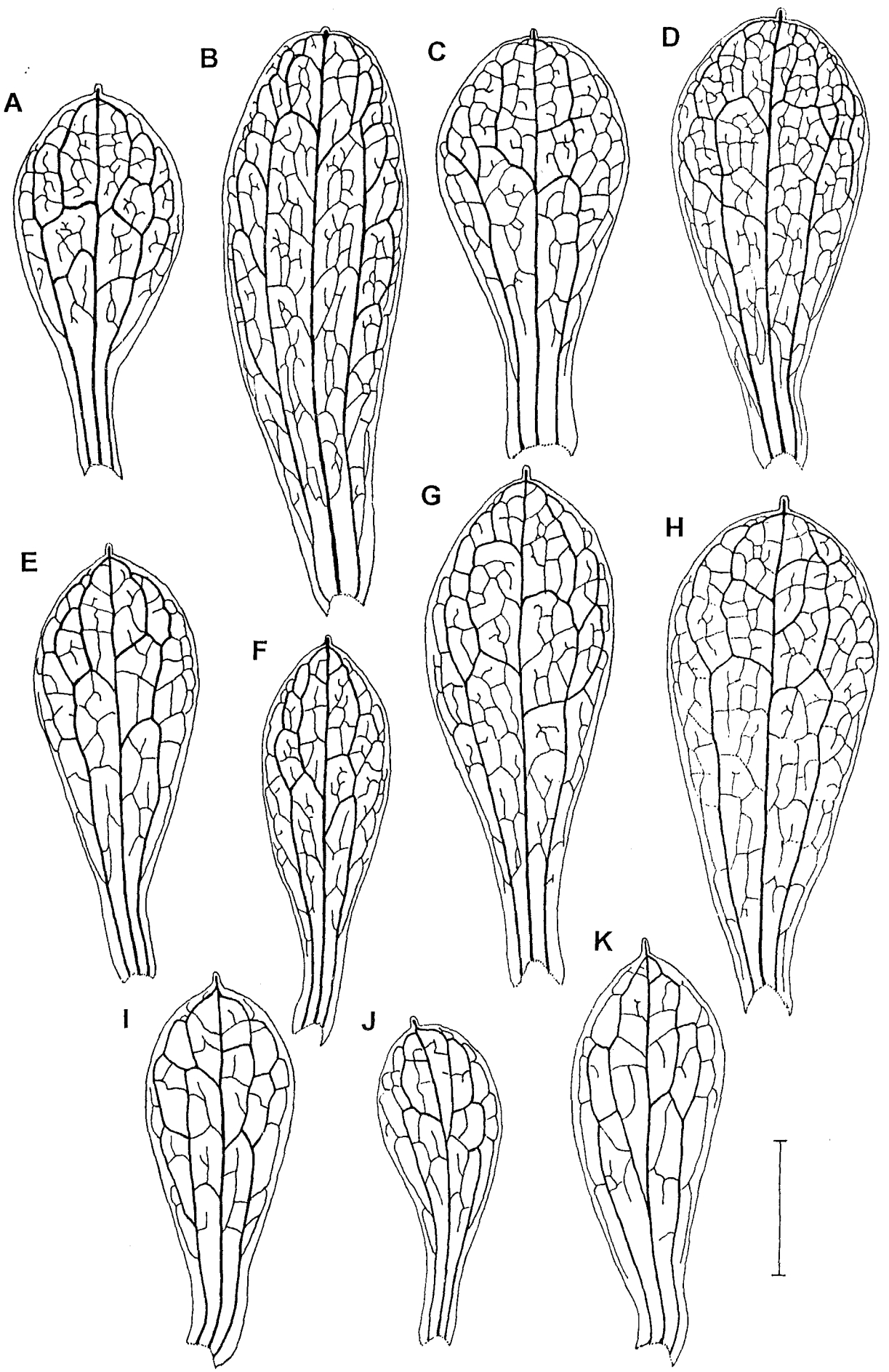
H, *W13.*

I, *S1.*

J, *S1* (a leaf from the juvenile phase).

K, *S2.*

Scale = 5 mm.


A
B
C
D
E
F
G
H
I
J
K

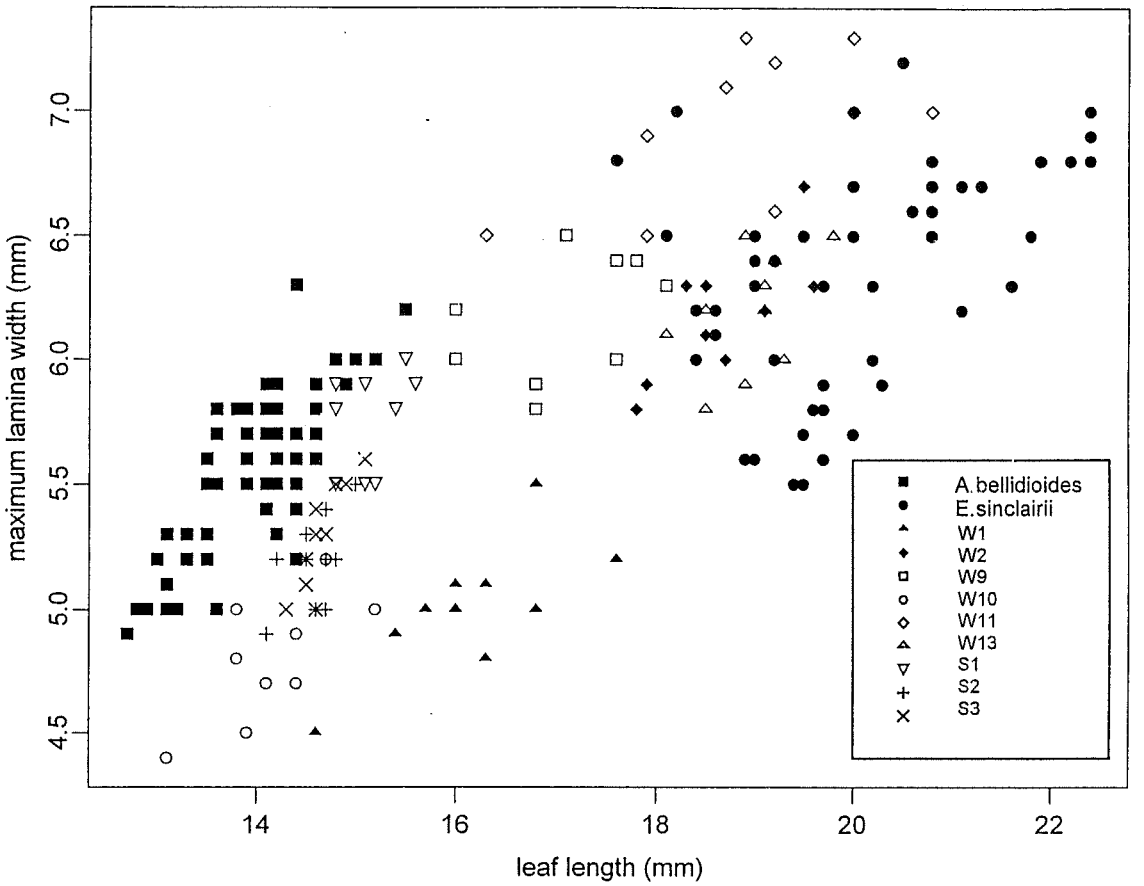


Figure 4.13. Leaf length and maximum lamina width in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

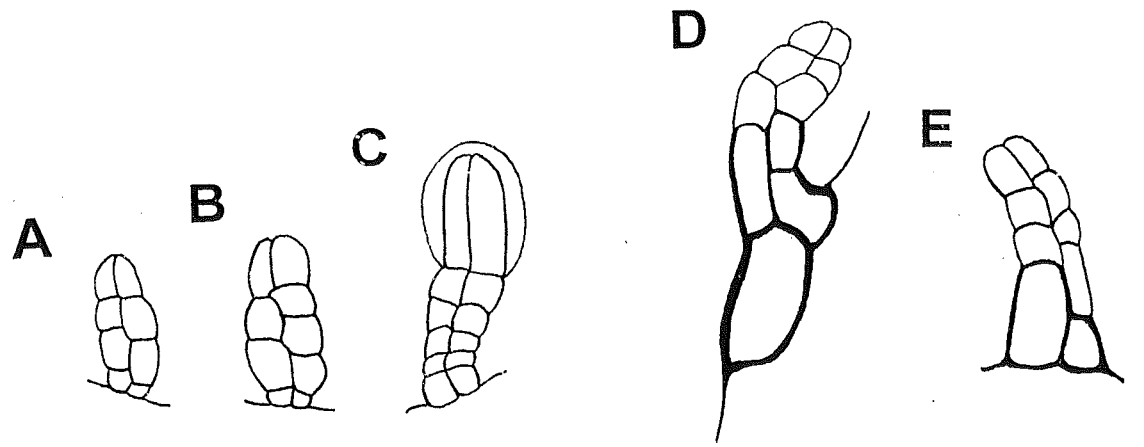


Figure 4.14. Glandular trichomes from leaves of *Anaphalioides bellidioides*, *Ewartia sinclairii* and *W13*. **A–C**, type A glandular trichomes from abaxial leaf surface; **D–E**, type B glandular trichomes from leaf margin. **A**, *A. bellidioides*; **B, *E. sinclairii*; C**, *W13*; **D**, ***A. bellidioides*; E**, ***W13***. Scale = 20 μm.

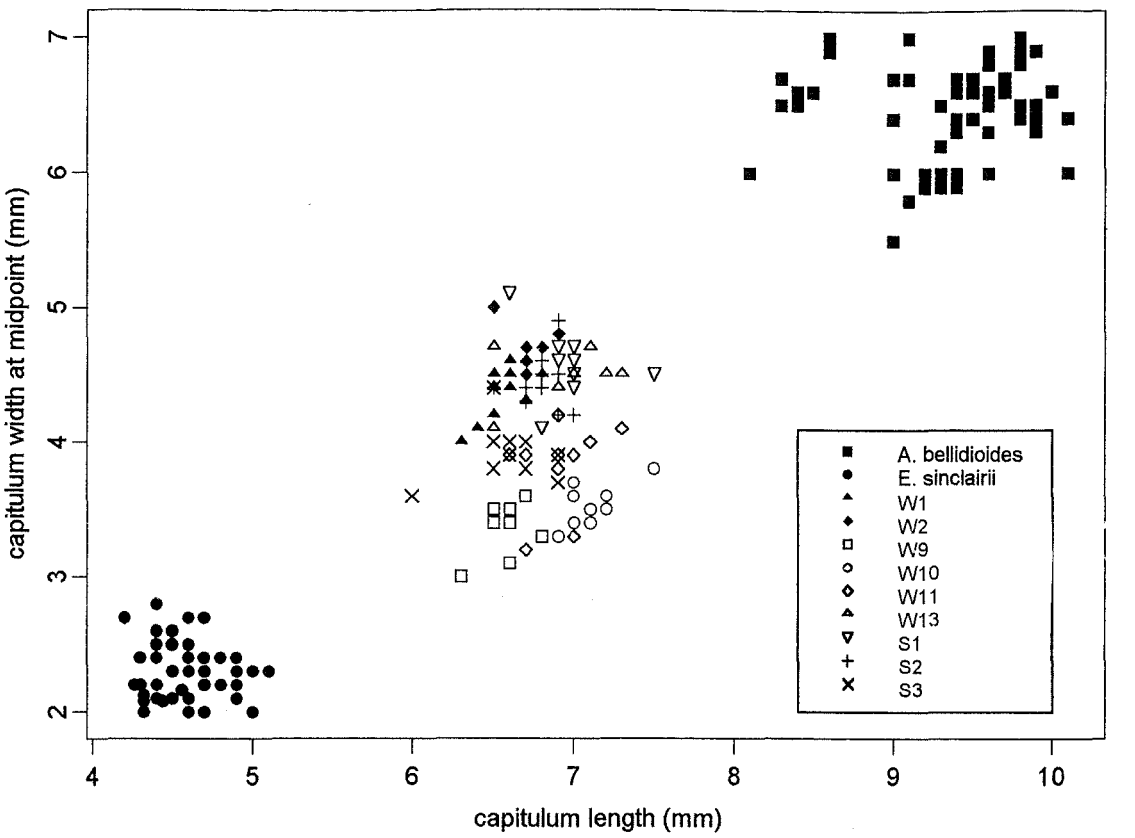


Figure 4.15. Capitulum length and width at the midpoint in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

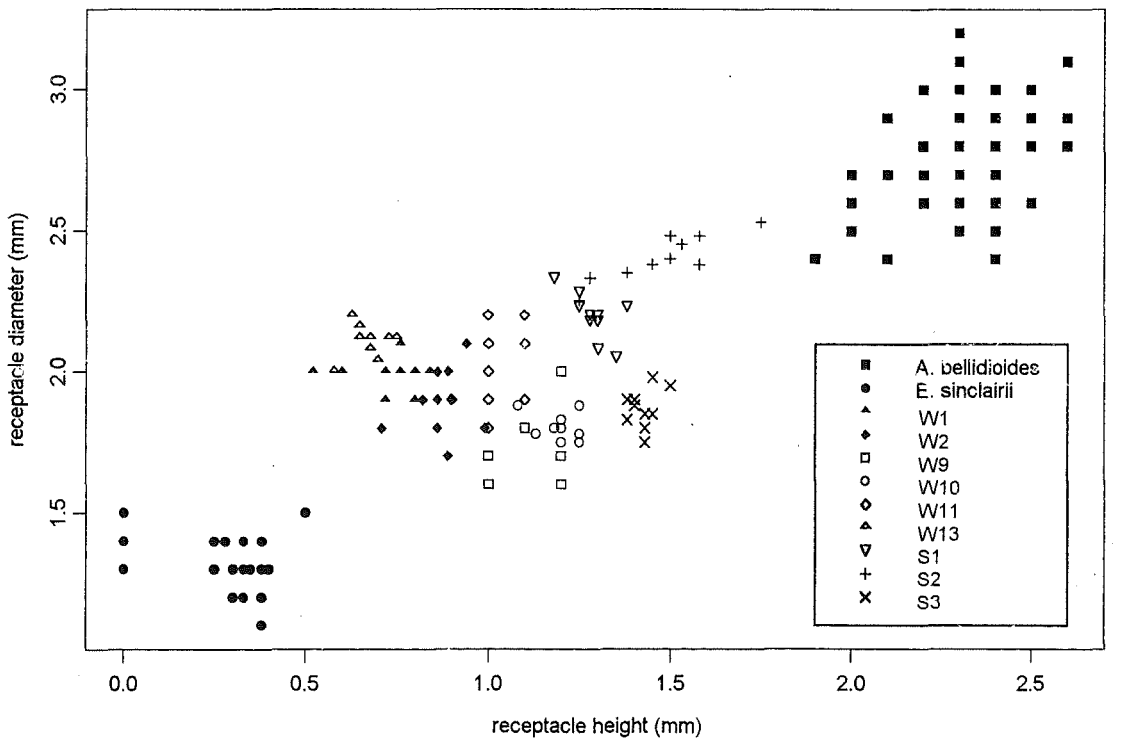


Figure 4.16. Receptacle height and diameter in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

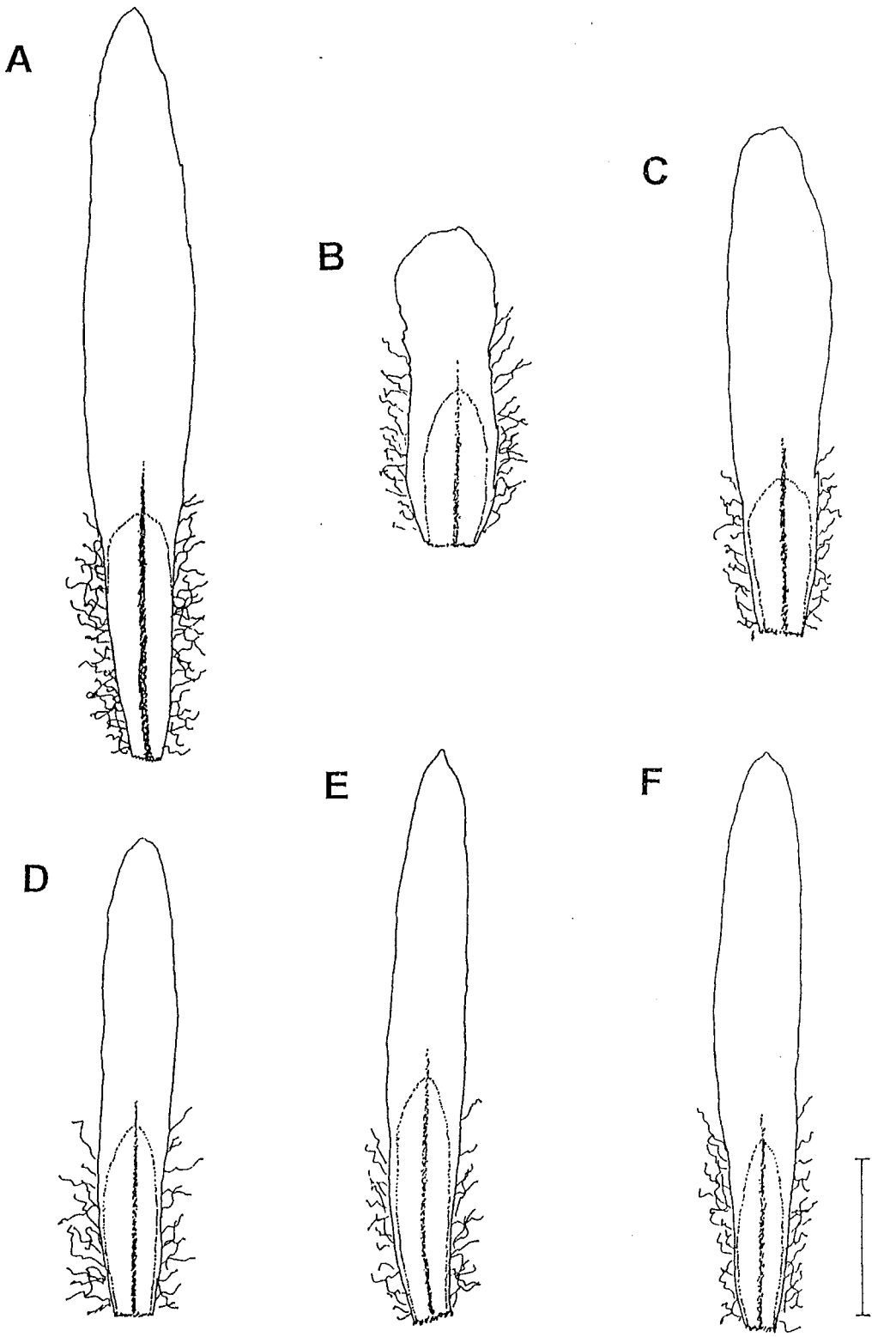


Figure 4.17. Inner involucral bracts of *Anaphalioides bellidioides*, *Ewartia sinclairii* and putative hybrids between the two species. A, *A. bellidioides*; B, *E. sinclairii*; C, *W13*; D, *W10*; E, *S2*; F, *S3*. Scale = 2 mm.

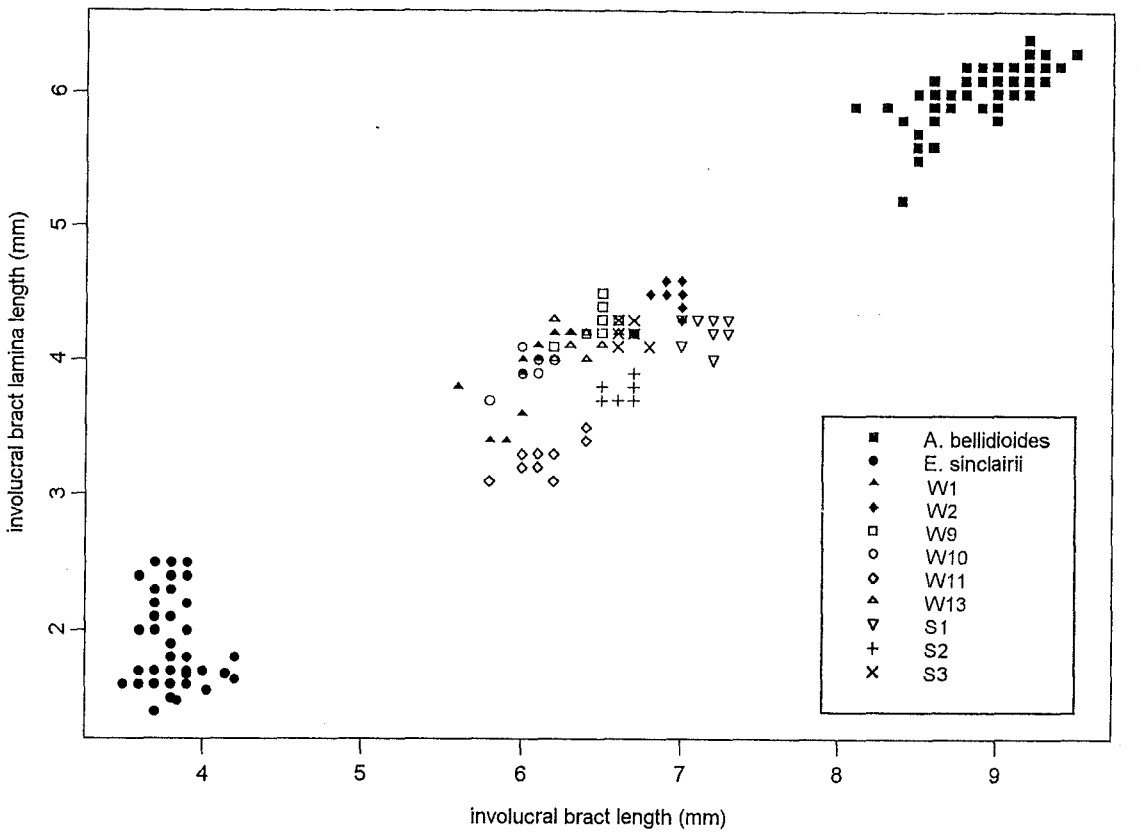


Figure 4.18. Total length and lamina length of inner involucral bracts in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

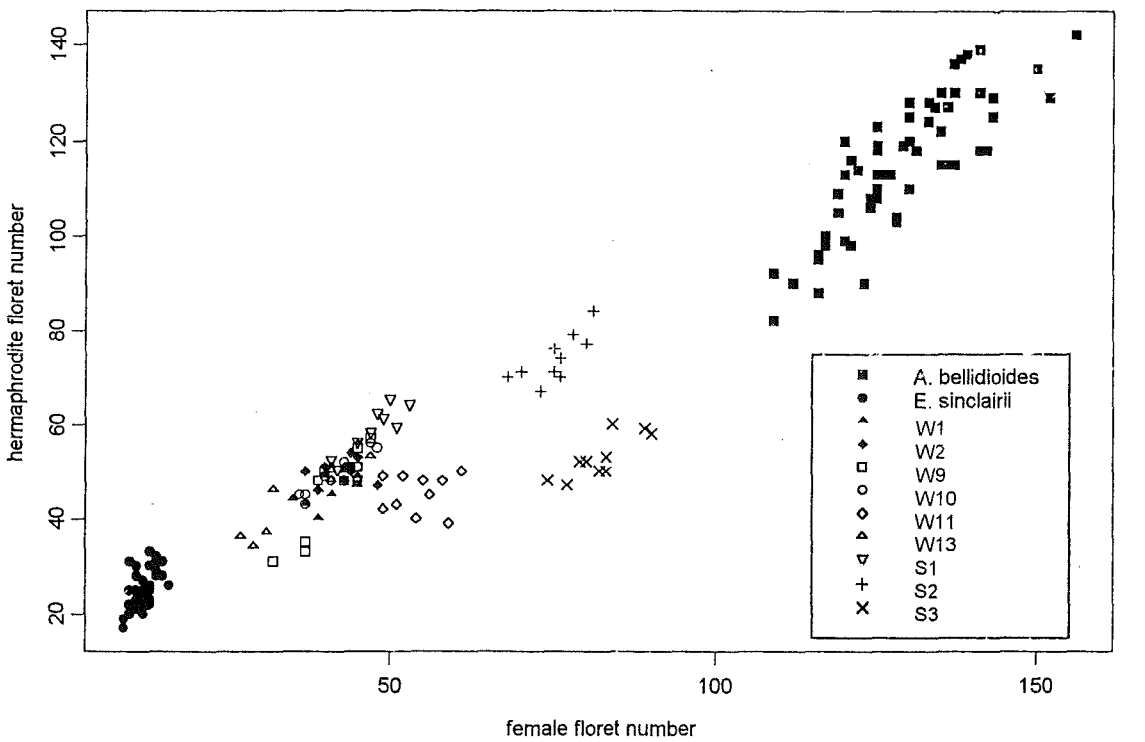


Figure 4.19. Number of female and hermaphrodite florets per capitulum in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

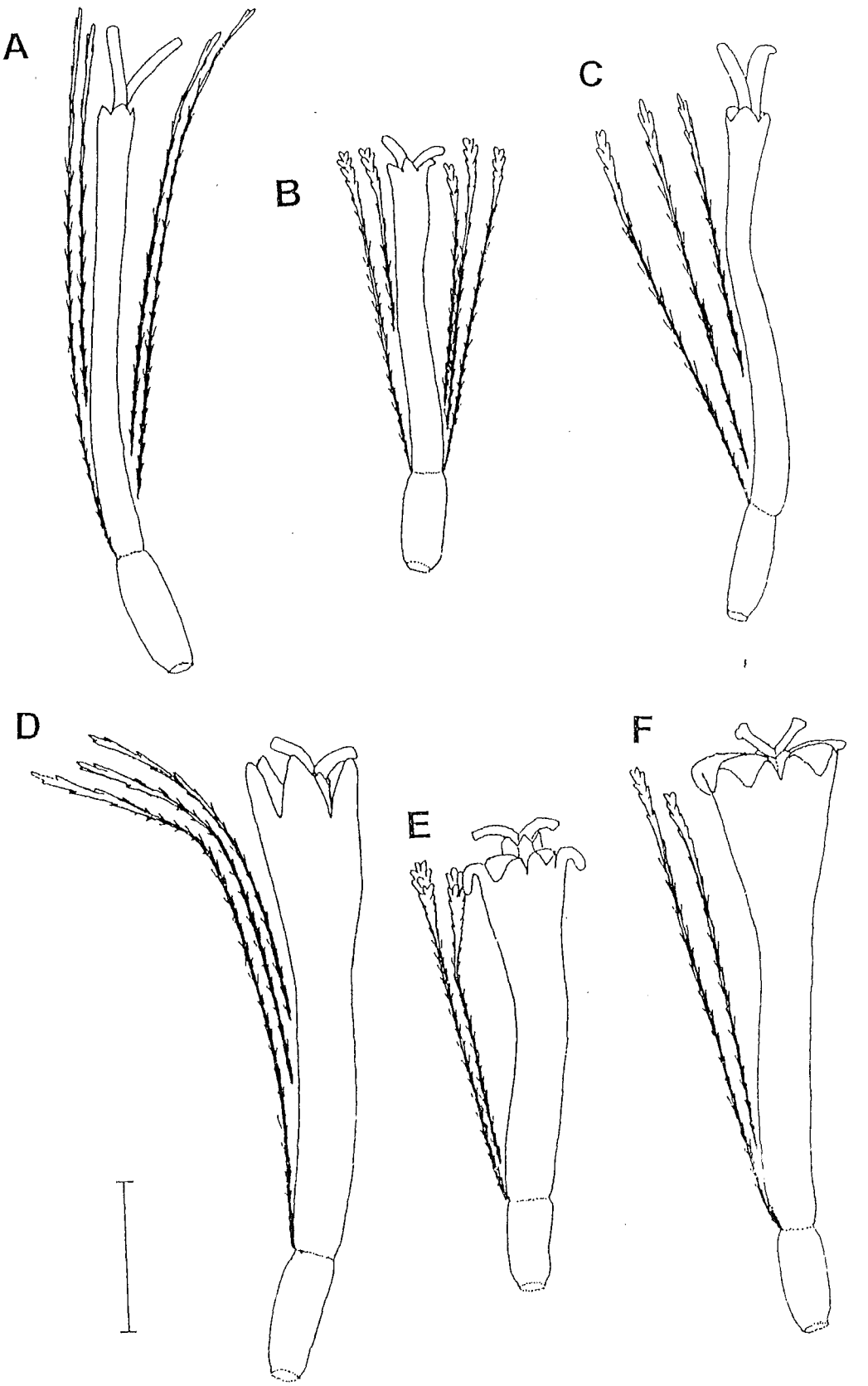


Figure 4.20. Female and hermaphrodite florets of *Anaphalioides bellidioides*, *Ewartia sinclairii* and *W11*. **A–C**, female florets; **D–F**, hermaphrodite florets. **A**, *A. bellidioides*; **B**, *E. sinclairii*; **C**, *W11*; **D**, *A. bellidioides*; **E**, *E. sinclairii*; **F**, *W11*. Scale = 1 mm. Note: not all pappus hairs are drawn for each floret.

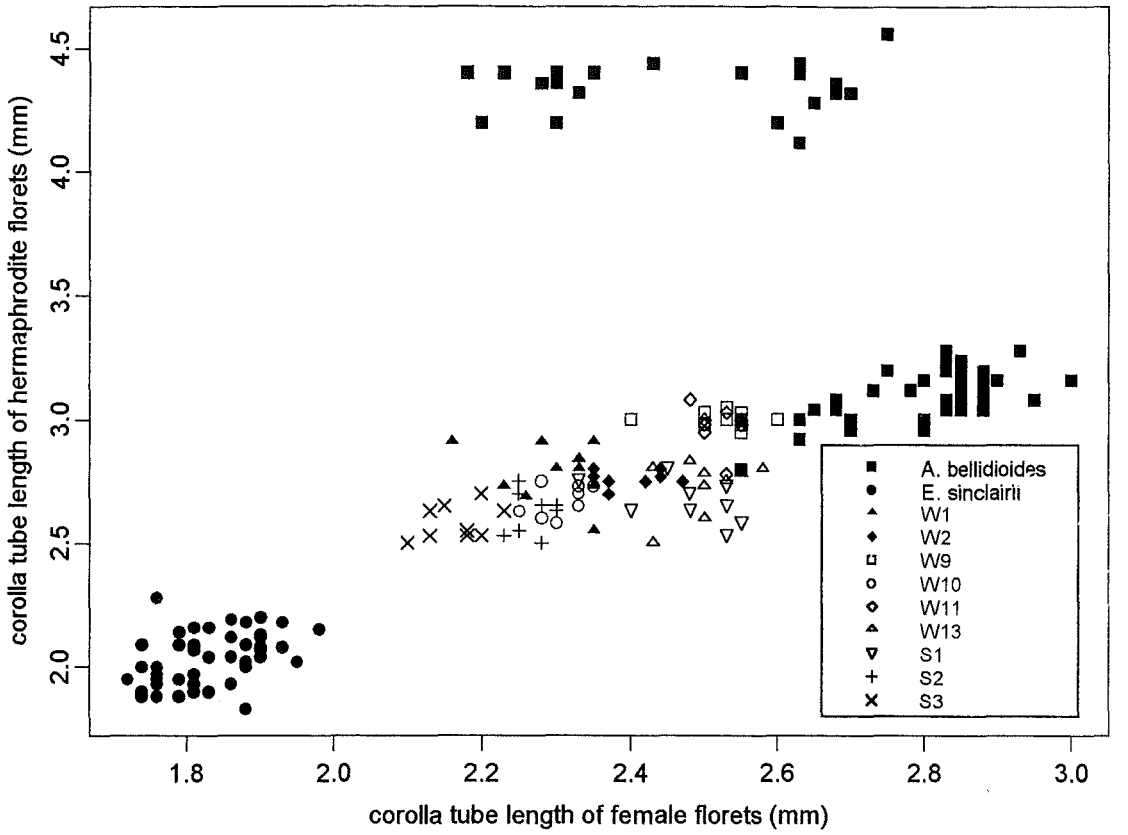


Figure 4.21. Corolla tube length of female and hermaphrodite florets in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

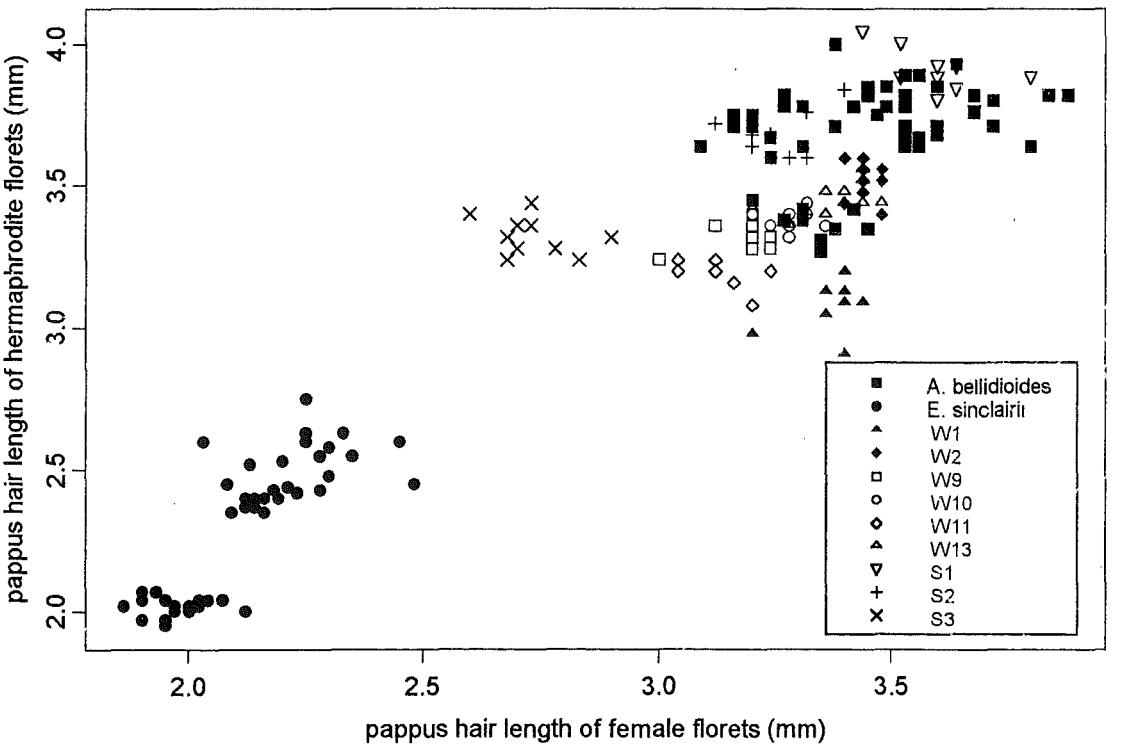


Figure 4.22. Pappus hair length of female and hermaphrodite florets in *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

Figure 4.23. Pappus hairs of *Anaphalioides bellidioides*, *Ewartia sinclairii* and *W2* (surface view).

A-H, pappus hair tip; **I-O,** pappus hair base.

A, *A. bellidioides*, from a female floret.

B, *A. bellidioides*, from a hermaphrodite floret.

C and D, *E. sinclairii*, from a female floret.

E, *E. sinclairii*, from a hermaphrodite floret.

F, *W2*, from a female floret.

G and H, *W2*, from a hermaphrodite floret.

I, *A. bellidioides*, from a female floret.

J, *A. bellidioides*, from a hermaphrodite floret.

K, *E. sinclairii*, from a female floret.

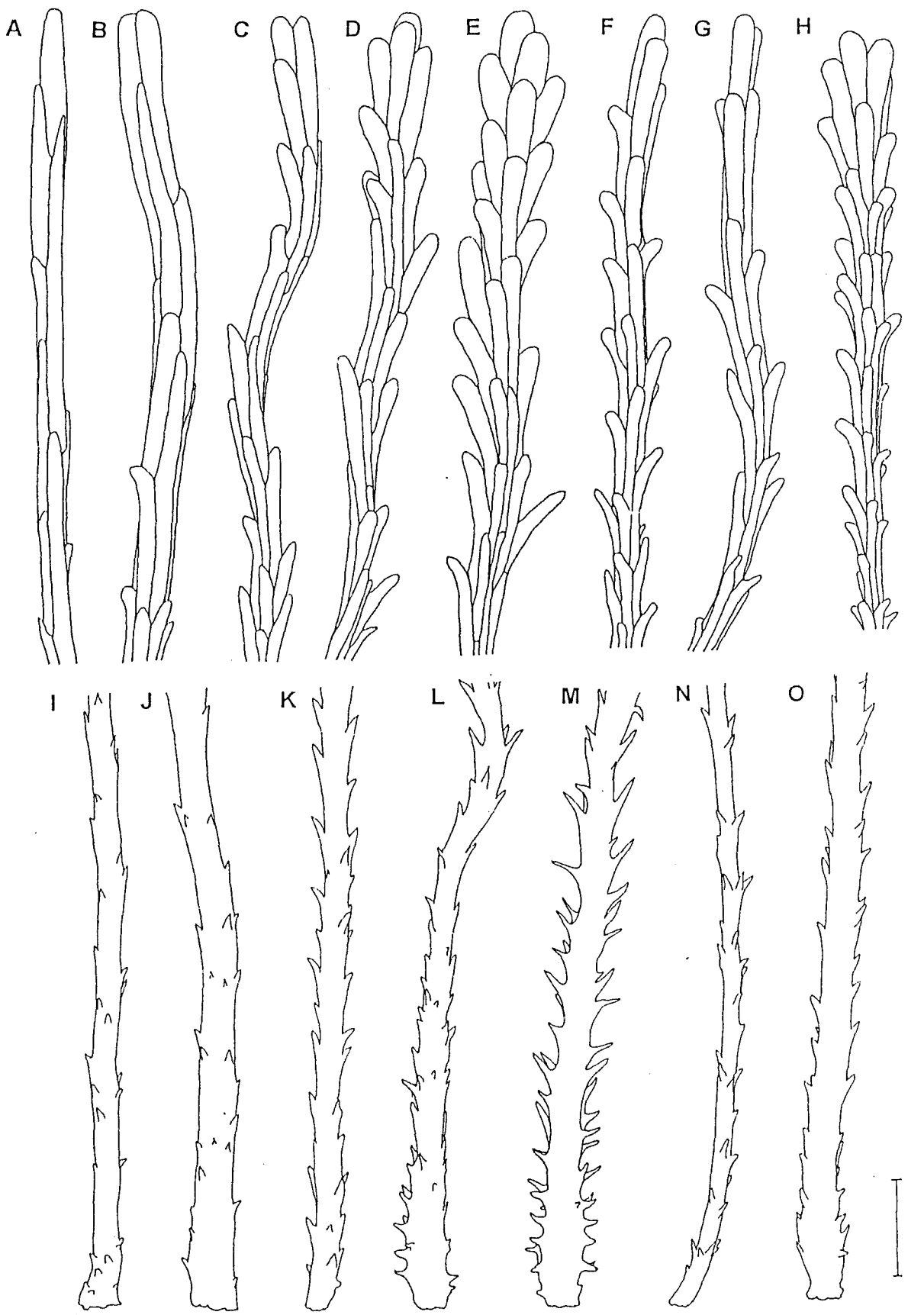
L, *E. sinclairii*, from a hermaphrodite floret.

M, *E. sinclairii*, from a female floret.

N, *W2*, from a female floret.

O, *W2*, from a hermaphrodite floret.

Scale = 100 μm.


A
B
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O

4.3.4.2 Analyses of morphological data

Character count

The method of determining the parental intervals for continuous characters (standard deviation or quartile intervals) had only a slight impact on classification of character states in the putative hybrids (Table 4.9–Table 4.12 pp. 161–162). The number of intermediate and extreme characters was slightly higher when quartiles were used, but overall patterns were identical. Extreme character states were rare and occurred principally in three continuous characters: maximum leaf width, mucro length, and the female:hermaphrodite floret ratio. In each instance, the putative hybrids fell within or very close to the range of variation in the putative parental species. The presence of a single multicellular twin hair on the ovary of a female floret, and the presence of occasional twin hairs on the ovary of hermaphrodite florets, in *S1* were the only novel characters recorded.

In the cultivated putative hybrids, discrete characters that discriminated *A. bellidioides* and *E. sinclairii* were predominantly classified as parental, whereas continuous characters were predominantly intermediate. Most of the field-collected putative hybrids possessed *A. bellidioides* and *E. sinclairii* parental character states in relatively equal proportions, but *W9* and *W10* possessed proportionally more (over 60 %) *A. bellidioides* characters than *E. sinclairii* characters. The field-collected putative hybrids possessed similar proportions of intermediate and parental character states. In contrast, parental characters appreciably outnumbered intermediate characters in the three seed-raised putative hybrids, with *A. bellidioides* characters predominant (over 70 %) over *E. sinclairii* characters. The character counts for *S2* and *S3* were virtually identical and *S1* differed principally in possessing two novel characters.

W4, *W8* and *W12* were unusual in that *E. sinclairii* characters outnumbered *A. bellidioides* characters. In *W12* the frequency of parental characters exceeded intermediate characters, but *W4*, *W5* and *W8* had relatively equal proportions of parental and intermediate characters. In all field-grown plants, discrete characters were predominantly classified as parental, whereas continuous characters were principally intermediate. Character counts for cultivated and field-grown clones of *W9* were very similar, but the proportion of parental characters relative to intermediate characters was slightly higher in the field-grown clone of *W10*.

Character index

The character index values (C-values) of the putative hybrids were intermediate between the putative parental species (Figure 4.24 p. 163). The type of characters (continuous, discrete or mixed) from which the character indices were calculated affected the C-values. In all individuals of *E. sinclairii*, *W5* and *W12* the C-values derived from continuous characters were notably higher than those derived from discrete characters, whereas in individuals of *A. bellidioides*, *W9* and *S1* the opposite was true. In most putative hybrids the difference in C-values was not marked. The relatively low continuous character C-values in *A. bellidioides* were due to the presence of extreme character values in some putative hybrids.

There was little variation in C-values among individuals of either species, but the putative hybrids were more variable with mixed-character C-values ranging from 0.7 for *S1* to 0.39 for *W8*. The mixed-character C-values for the three seed-raised putative hybrids (0.62–0.7) were slightly higher than for the other cultivated putative hybrids (0.49–0.59), among which *W9* (0.59) and *W10* (0.57) had the highest C-values. Mixed-character C-values were similar among the field-grown putative hybrids (0.39–0.49).

The underlying character frequency distributions for continuous and discrete characters were very different for the cultivated putative hybrids (Figure 4.25 p. 164). The frequency distributions exhibited similar patterns to the character counts. Parental character states in the putative hybrids were much more frequent for discrete characters than for continuous characters, for which intermediate values predominated. The same trends existed for the field-grown putative hybrids (data not presented). The preponderance of *A. bellidioides* characters in the seed-raised putative hybrids, and to a lesser extent in *W9* and *W10*, was also evident. The frequency distribution of continuous characters varied between the putative hybrids and there was no clear difference between the field-collected and seed-raised putative hybrids. Despite the marked differences in frequency distributions of continuous and discrete characters, the C-values derived from the two data sets were similar overall, as already discussed.

Greater variation in continuous characters among the field-grown plants was evident in the character indices. C-values derived from continuous characters were lower in most *A. bellidioides* plants and higher in many *E. sinclairii* plants (as compared with cultivated plants) owing to extreme values in some putative hybrids. However, for both species C-values derived from mixed characters were similar between the field-grown and cultivated plants.

The C-value for *W12* derived from continuous characters (0.65) placed it very close to *A. bellidioides*, but this was principally because *W12* received the maximum value for four characters (leaf length, maximum lamina width and corolla tube length in female and hermaphrodite florets). Exclusion of these characters reduced the C-value to 0.46. Similarly, *S1* received the maximum value for two discrete characters (presence of glandular trichomes on the ovary of female florets, and presence of twin hairs on the ovary of hermaphrodite florets). Exclusion of these characters reduced its discrete-character C-value only marginally to 0.73.

Separate indices were calculated from field-grown and cultivated clones of *W9* and *W10*. For all data sets, the C-values for the field-grown clones were slightly lower than for the cultivated clones, but the patterns between data sets were identical. For *W9* the continuous-character C-values were lower than the discrete-character C-values, whereas for *W10* C-values derived from continuous and discrete characters showed little difference. Overall, C-values for field-grown specimens of *W9* and *W10* were slightly higher than for *W4*, *W5* and *W8*. The C-values derived from mixed data were identical to that of *W12*, but as discussed above, there was considerable variation between indices calculated from discrete and continuous characters among these plants.

The inclusion of ratios had a negligible impact on the character indices (data not presented). C-values were marginally lower in most of the putative hybrids, and raised marginally in two putative hybrids, but the overall patterns were unaffected. Exclusion of the two characters unique to *S1* marginally reduced the C-value of *S1* and slightly raised the C-values for individuals of *A. bellidioides*, but did not affect the overall patterns.

Cluster analysis

Agglomerative hierarchical clustering generated phenograms of very similar structure with all linkage methods. Three clusters of OTUs were clearly differentiated by all methods: *A. bellidioides*, *E. sinclairii* and the putative hybrids (Figure 4.26 p. 165). The cluster of putative hybrids was always linked to the *A. bellidioides* cluster. The putative parental species exhibited little intraspecific variation with individuals exhibiting dissimilarities of less than 0.1 in all analyses. The putative hybrids were more dissimilar and variable than the species clusters. The seed-raised and field-collected putative hybrids consistently formed separate clusters (with a dissimilarity of 0.27 with group-average linkage). *W2* and *W13* were the most similar of the putative hybrids and were linked by each linkage method. *W9* and *W10* were

clustered by average, single and weighted-average linkage. *W1* and *W11* were linked separately to the *W2/W13* cluster with group-average, single and weighted-average linkage, but formed a separate cluster (with a dissimilarity of 0.1) with complete linkage. With each linkage method *S2* and *S3* were clustered and *S1* was the most dissimilar of all of the putative hybrids (e.g., it was linked to the other putative hybrids at 0.185). Linkage values were highest with complete linkage (in which the highest dissimilarity was 0.91) and lowest with single linkage (in which the greatest dissimilarity was 0.44). For each linkage method six randomisations of the OTU order returned a phenogram of identical structure as the default.

The overall Spearman's rank and Pearson's product-moment correlation coefficients for the agglomerative clustering phenograms were 0.94 and 0.89–0.9 respectively for each linkage method, indicating a good fit existed between the original dissimilarities and the phenogram for each method. The correlation coefficients declined sharply among the highest linkages. With group-average linkage, the lowest correlation coefficients for a linkage was 0.62 and 0.76 (Figure 4.27 p. 166). With complete linkage, the junction of two *A. bellidioides* clusters possessed the lowest correlation coefficients (0.54 and 0.63 respectively). The correlation coefficients were higher with increasing dissimilarity and stabilised at 0.9–0.95 for each method. With group-average linkage the correlation coefficients for all of the putative hybrids' linkages were 0.88–0.97 for the Pearson's coefficient and 0.86–0.97 for the Spearman's coefficient. Excluding the terminal linkages, in all methods the linkage between the field-collected and seed-raised putative hybrids had the highest correlation coefficients.

OTU-based jackknife analysis provided 100 % support for most linkages in the agglomerative-clustering phenograms. Among the putative hybrids, only the junction points of *W1* (89.5 % support with average linkage, 94.7 % with single linkage and 82.4 % with complete linkage) and *W11* (84.2 %, 94.7 % and 89.5 % respectively) had lower jackknife support values. In the *A. bellidioides* and *E. sinclairii* clusters two junctions received 94.1 % or 94.7 % support values with average and single linkage, and four junctions had support values ranging from 88.2 % to 94.7 % with complete linkage.

Character-based jackknife analysis of the agglomerative-clustering phenograms provided high support for most of the lowest dissimilarities for each linkage method. Among the putative hybrids, the linkage between *W2* and *W13* received 96.2 % support with average and single linkage. The clustering of *W9* and *W10* received 90.4 % support with average linkage and 84.6 % support with single linkage. The junction points of *W11* received 90.4 % and 90.3 %

wih average and single linkage respectively, while the linkages of *W1* received 90.4 % and 94.2 % support. All other linkages involving putative hybrids received 100 % support.

The field-grown putative hybrids formed a single, hierarchical group linked to the *A. bellidioides* cluster with group-average linkage (Figure 4.28 p. 167). *W4* and *W8* were the most similar plants followed by, with increasing dissimilarity, *W9*, *W5*, *W10* and *W12*. The overall correlation coefficients for the phenogram were 0.89 and 0.88 for the Spearman's and Pearson's coefficients respectively. Pearson's product-moment correlation coefficients for the putative hybrids' linkages ranged from 0.76 for the *W4–W8* linkage to 0.94 for the *W12* linkage (Figure 4.29 p. 168). Spearman's rank correlation coefficients for the same linkages were 0.77 and 0.89. Six randomisations of the OTU order returned a single phenogram with the same structure. Single, complete and group-weighted linkage returned phenograms with the same cluster structure for the putative hybrids. With single linkage, OTUs were represented as being more similar (e.g., the *W12* linkage was 0.12) and the correlation coefficients for the higher linkages were reduced (e.g., Pearson's coefficient for the *W4–W8* linkage was 0.62), With complete linkage, OTUs were more dissimilar (e.g., the *W12* linkage height was 0.22) and correlation coefficients slightly higher than for single linkage. However, the overall correlation coefficients for the phenogram were almost identical for each method.

Fuzzy partitioning of cultivated plants yielded normalised Dunn's partition coefficients of 0.63 for a three-cluster analysis and 0.60 for a four-cluster analysis. The average silhouette width was 0.72 with three clusters and 0.75 with four clusters. Individuals of *A. bellidioides* and *E. sinclairii* formed separate clusters with high membership coefficients (0.82 or above) and average cluster widths (0.89-0.93) in both analyses. The putative hybrids formed a single cluster (average silhouette width 0.49) in the three-cluster partitioning. The field-collected putative hybrids had membership coefficients of 0.76-0.85 for the third cluster, whereas the seed-raised putative hybrids had considerably lower membership coefficients for this cluster (0.48-0.55), only slightly higher than their membership coefficients (0.30-0.37) for the *A. bellidioides* cluster. Of the putative hybrids *S1* had the lowest coefficient for membership of the third cluster and the highest for membership of the *A. bellidioides* cluster. In the four-cluster partitioning, the field-collected and seed-raised putative hybrids formed separate clusters. The cluster of field-collected putative hybrids had an average silhouette width of 0.61. The membership coefficient of *W2* for this cluster was 0.82 but for all other field-collected putative hybrids the coefficient ranged from 0.61 (*W9*) to 0.71 (*W11*). The cluster of seed-raised putative hybrids had an average silhouette width of 0.44. *S2* and *S3* had relatively

high membership coefficients for the fourth cluster of 0.82 and 0.77 respectively. *S1* was less confidently placed and had membership coefficients of 0.49 for cluster four and 0.23 for cluster three.

Fuzzy partitioning of field-grown plants yielded normalised Dunn's partition coefficients of 0.57 for a three-cluster analysis, 0.37 for a four-cluster analysis and 0.34 for a five-cluster analysis. The average silhouette width was 0.73 with three clusters and 0.44 with four clusters. In the three-cluster partitioning the putative hybrids formed a single cluster with an average silhouette width of 0.50. Membership coefficients for the putative hybrids ranged from 0.57 (*W12*) to 0.79 (*W8*). With four clusters, the putative hybrids still formed a single cluster (average silhouette width 0.50) and plants of *E. sinclairii* were divided between two clusters. Membership coefficients for the putative hybrids for the fourth cluster were little changed (0.45–0.72). Membership coefficients for plants of *E. sinclairii* in the second and third clusters were relatively low (0.45–0.48). A five-cluster partitioning resulted in *W5* and *W12* forming a separate cluster (average silhouette width 0.2) with membership coefficients of 0.79 (*W5*) and 0.44 (*W12*). The other putative hybrids formed a cluster with an average silhouette width of 0.37 and membership coefficients ranging from 0.42 (*W10*) to 0.63 (*W4*). The average silhouette width in this analysis was 0.38.

In partitioning around medoids of cultivated plants, the maximum average silhouette width (0.75) was obtained for the four-cluster partitioning, but the coefficient was only marginally lower in three- and five-cluster partitionings (0.72 and 0.73 respectively). The putative parental species formed tight, well separated clusters and the silhouette widths for individuals of each species were greater than 0.84. The field-collected and seed-raised putative hybrids were separated in the four-cluster analysis with *W2* and *S2* as the medoids. All four groups were L* clusters (i.e., the cluster's diameter was smaller than its separation from other clusters). Based on its silhouette widths, *S1* was the most isolated putative hybrid and in the five-cluster analysis formed an isolated cluster. Average silhouette width, cluster diameter and within-cluster dissimilarities were reduced with increased splitting of the putative hybrids, but cluster separation was also reduced. The silhouette widths of the field-collected putative hybrids were similar whether three, four or five clusters were specified. *W9* and *W10* had the lowest silhouette widths and *W2* the highest, but there was very little variation among the field-collected putative hybrids. The silhouette widths of *S2* and *S3* were highest when these individuals formed a separate cluster in the k = 5 partitioning.

With partitioning around medoids of field-grown plants, the average silhouette width was highest (0.73) for a three-cluster partitioning and declined with increasing cluster number. In each analysis the putative parental species formed L* clusters but the putative hybrids never formed L* clusters. In a three-cluster partitioning, the putative hybrids formed a single cluster. The silhouette widths were lowest for *W10* (0.35) and *W12* (0.43), whereas the silhouette widths for the other putative hybrids ranged from 0.5 (*W9*) to 0.61 (*W5*). When four clusters were specified, the putative hybrids were divided into two clusters of which *W8* and *W12* were the medoids. *W10* was separated from the putative-hybrids cluster in a five-cluster analysis and *W5* with six clusters specified. For each putative hybrid, the silhouette widths declined sharply with increasing number of clusters.

HYWIN

HYWIN analysis of all OTUs generated 3420 hypotheses for evaluation. Only the 114 highest-ranked combinations (representing the 0.95 probability that all OTUs would be ranked as a hybrid) were evaluated. In all analyses of complete data sets, no individual of *A. bellidioides* or *E. sinclairii* was hypothesised to be a hybrid, and no putative hybrid was predicted to be a parent of another OTU, in the 114 highest-ranked combinations. Without exception, a putative hybrid was always predicted to be a hybrid between an individual of *A. bellidioides* and an individual of *E. sinclairii* (Table 4.13 p. 169).

Analysis of continuous characters with the default weightings resulted in all of the field-collected putative hybrids and *S3* ranked as hybrids among the 114 highest-ranked combinations. *W1*, *W2* and *W13* were the highest and most frequently ranked as hybrids. If intermediacy received a low weighting (wI = 0.1, wE = 1, wP = 1), *W11* was ranked higher and more frequently, and *S3* was not ranked at all, compared to the results with the default weightings. Reducing the parental-distance weighting (wI = 1, wE = 1, wP = 0.1) had little impact on the rankings. Lowering the equality weighting had the greatest impact on the results. Most notably, the seed-raised putative hybrids were frequently ranked. When intermediacy and parental distance received maximal weighting (wI = 1, wE = 0.1, wP = 1), all of the putative hybrids except *W11* were suggested to be hybrids among the 114 highest-ranked combinations. Giving equality and parental distance low weightings and intermediacy maximal weighting (wI = 1, wE = 0.1, wP = 0.1) yielded similar results. If intermediacy and equality received low weights (wI = 0.1, wE = 0.1, wP = 1) all of the putative hybrids were suggested to be hybrids among the 16 highest-ranked combinations (Table 4.14 p. 170).

Analysis of discrete characters separately resulted in multiple combinations receiving identical hybrid optimality scores, so the predictive value of the rankings was greatly reduced. With the default weightings, *W1*, *W2*, *W11* and *W13* were the only putative hybrids to be ranked within the 0.95 probability level.

In an analysis of the mixed-character data set using the default weights (wI = 1, wE = 1, wP = 1), *W1* and *W13* received the 36 highest rankings and, together with *W2* and *W11*, filled the 111 highest combinations. *W10* was ranked 112th and 114th. *W9* and the three seed-raised putative hybrids were not ranked among the 114 most likely hypotheses. Reducing the equality weighting (wI = 1, wE = 0.1, wP = 1) again had the greatest impact; all of the field-collected putative hybrids were ranked within the 70 highest rankings but none of the seed-raised putative hybrids were ranked within the 0.95 probability level. *W1*, *W2* and *W13* were the highest-ranked putative hybrids. When intermediacy also received a low weighting (wI=0.1, wE=0.1, wP=1), all of the field-collected hybrids were ranked within the 26 highest-ranked hypotheses and *S3* was ranked once as a hybrid. Reducing the parental-distance weighting (wI = 1, wE = 1, wP = 0.1) had a minimal impact on the rankings compared to results with the default weightings. Following the exclusion of *W1*, *W2*, *W11* and *W13*, the data set was reanalysed with the default weights and the 87 highest rankings (representing the 0.95 probability level) were evaluated. *W10* and *W11* filled the 60 highest rankings and *S2* and *S3* were also ranked as hybrids. Only *S1* was not ranked as a hybrid.

Examination of the equality scores in each analysis indicated that *W1*, *W2*, *W11* and *W13* tended to be equidistant between the hypothesised parents, whereas the seed-raised putative hybrids were closer to *A. bellidioides* than *E. sinclairii*.

Metric multidimensional scaling

Thirteen positive eigenvalues were obtained. The first principal-coordinate axis accounted for 87.6 % of the total variation. The second and third axes explained a further 3.6 % and 3.0 % respectively. The 'sum of squared distances' stress values for the first three axes were 0.0036, 0.0018 and 0.0007 respectively. For both measures, values declined sharply with increasing number of dimensions. Three well-separated groups of OTUs (*A. bellidioides*, *E. sinclairii* and the putative hybrids) were distinguished on the first axis (Figure 4.30 p. 171). The putative parental species were widely separated and formed particularly tight clusters on the first axis, whereas the putative hybrids were less tightly clustered. The second axis principally separated the seed-raised and field-collected putative hybrids, but one *A. bellidioides* OTU

was also segregated. The third axis accounted for greater within-group variation among *E. sinclairii* and the putative hybrids, and was uninformative regarding discrimination of groups. *S1* was isolated from all other OTUs on the third axis. The field-collected putative hybrids clustered on the first two axes, but *W1*, *W2* and *W13* were slightly separated on the third axis. The seed-raised putative hybrids also clustered on the first two axes, but the three individuals were separated on the third axis. Minimal spanning trees supported the groupings on the first two axes and the absence of any well-defined groups on the third axis (Figure 4.31 p. 172).

Reducing the number of putative hybrids included in the data set had a negligible impact. Data sets containing the putative parents with *W2* alone, *S1* alone, or *W2*, *S1* and *S2* were analysed. In each instance, the putative hybrids were intermediate on the first axis but the single hybrid, or the most divergent hybrid, was more extreme than OTUs of the putative parents on the second axis. Group resolution was poor on the third axis. The proportion of the total variation explained by the first axis increased with reducing putative-hybrid number (up to 96.7 % with only *W2* included in the data set).

Split decomposition

Split decomposition of the complete data set identified 42 weakly compatible split systems. The splits graph had a fit of 76.8 % (Figure 4.32 A–B p. 173). The putative hybrids were placed intermediate between the putative parental species, but were closer to *A. bellidioides*. A compatible split separated the field-collected and seed-raised putative hybrids (Figure 4.32 C–D). The seed-raised putative hybrids were placed closer to *A. bellidioides* than the field-collected putative hybrids. Compatible splits were absent within the two putative-hybrid groups. For all of the putative hybrids the terminal edges were short (0.0512 or less), of which *S1* had the longest edge. A Buneman tree comprising 24 compatible split systems supported the distinction of four groups (Figure 4.32 C & D).

The putative parental species were excluded from the data set and the dissimilarities reanalysed to investigate relationships among the putative hybrids. The splits graph contained 20 weakly compatible splits and had a fit of 98.2 % (Figure 4.32 E). A Buneman tree comprised 12 compatible splits and had a fit of 74.6 % (Figure 4.32 F). Weakly compatible split systems linked all of the putative hybrids. Compatible splits separated the field-collected and seed-raised putative hybrids, and *S1* from *S2* and *S3* (Figure 4.32 F). *S1* had the longest terminal edge. The field-collected putative hybrids were represented as being extremely similar, but a short compatible split linked *W2* and *W13*.

Putative hybrid	Parental					Intermediate		Extreme	Novel
	A. bellidioides		*E. sinclairii*		Total				
	D	C	D	C		D	C	C	D
W1	13	2	14	1	30	10	16	0	0
W2	15	1	12	2	30	10	16	0	0
W9	18	2	9	1	30	10	15	1	0
W10	17	2	11	1	31	9	15	1	0
W11	15	3	13	1	32	9	13	2	0
W13	13	2	15	3	33	9	14	0	0
S1	25	4	8	0	37	4	14	1	2
S2	24	3	11	0	38	2	15	1	0
S3	24	3	11	0	38	3	15	1	0

Table 4.9. Character counts for the cultivated putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii* using standard deviation intervals. Only characters discriminating the parental species were included. C, continuous characters; D, discrete characters.

Putative hybrid	Parental					Intermediate		Extreme	Novel
	A. bellidioides		*E. sinclairii*		Total				
	D	C	D	C		D	C	C	D
W1	13	1	14	1	29	10	16	1	0
W2	15	1	12	2	30	10	16	0	0
W9	18	0	9	1	28	10	17	1	0
W10	17	1	11	0	29	9	17	1	0
W11	15	0	13	0	28	9	16	3	0
W13	13	1	15	2	31	9	16	0	0
S1	25	1	8	0	34	4	15	3	2
S2	24	2	11	0	37	2	15	2	0
S3	24	2	11	0	37	3	15	2	0

Table 4.10. Character counts for the cultivated putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii* using quartile intervals. Only characters discriminating the parental species were included. C, continuous characters; D, discrete characters.

Putative hybrid	Parental					Intermediate		Extreme	Novel
	A. bellidioides		*E. sinclairii*		Total				
	D	C	D	C		D	C	C	
W4	6	3	9	1	19	6	11	0	0
W5	3	2	7	0	12	3	11	0	0
W8	5	2	10	0	17	6	13	0	0
W9	15	2	11	1	29	11	12	0	0
W10	15	3	13	0	31	9	12	0	0
W12	11	3	15	0	29	7	8	2	0

Table 4.11. Character counts for field-grown putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii* using standard deviation intervals. Only characters discriminating the parental species were included. Four characters were excluded owing to overlapping standard deviation intervals of the two species: maximum lamina width; mucro length; female-floret corolla tube length; and hermaphrodite-floret corolla tube length. C, continuous characters; D, discrete characters.

Putative hybrid	Parental					Intermediate		Extreme	Novel
	A. bellidioides		*E. sinclairii*		Total				
	D	C	D	C		D	C	C	
W4	6	2	9	2	19	6	13	1	0
W5	3	4	7	0	14	3	11	0	0
W8	5	2	10	1	18	6	15	0	0
W9	15	2	11	1	29	11	13	2	0
W10	15	4	13	1	33	9	13	0	0
W12	11	3	15	0	29	7	9	4	0

Table 4.12. Character counts for field-grown putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii* using quartile intervals. Only characters discriminating the parental species were included. Maximum lamina width was excluded owing to the overlapping quartile intervals of the two species. C, continuous characters; D, discrete characters.

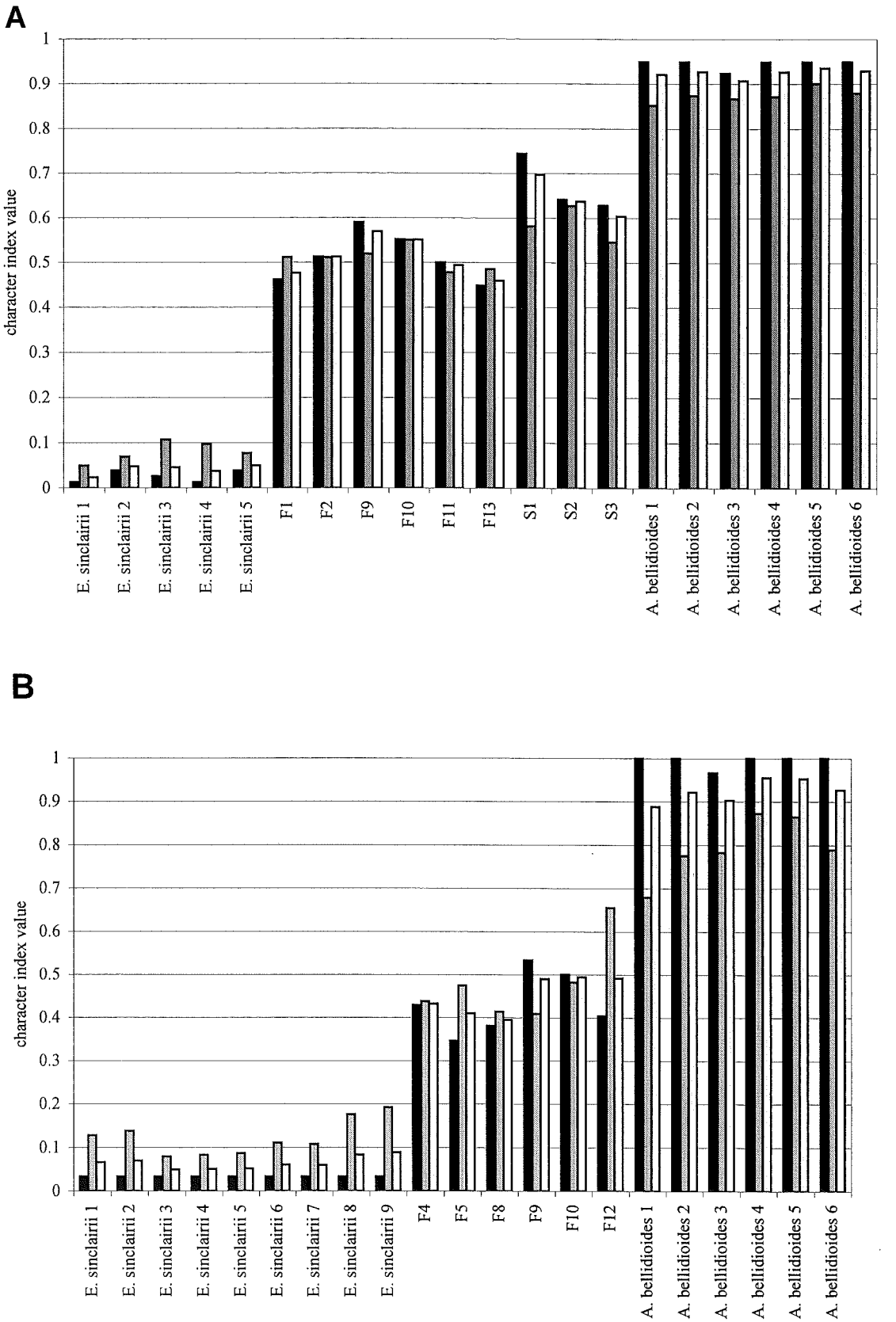
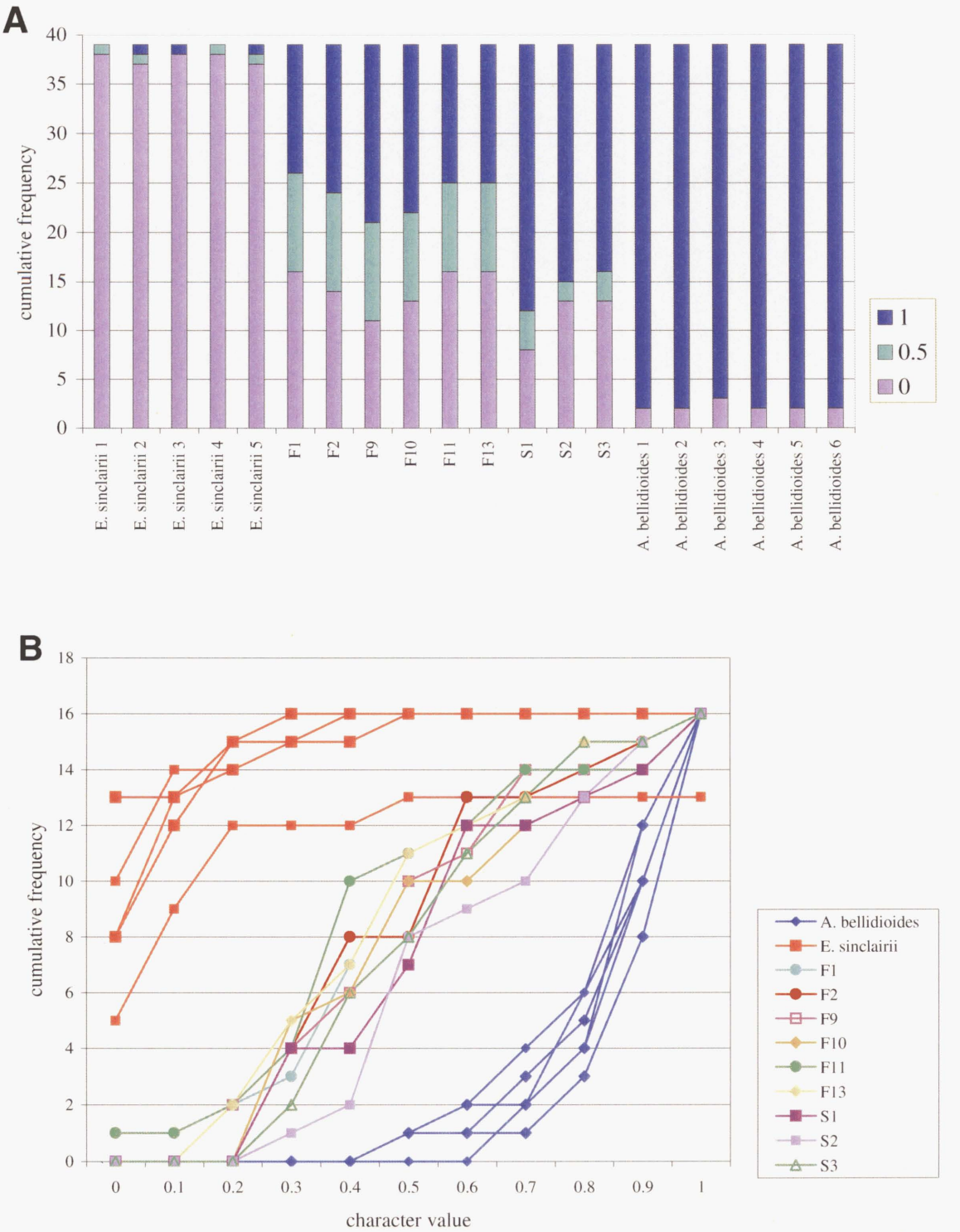


Figure 4.24. Character indices for *A. bellidioides*, *E. sinclairii* and putative hybrids between the two species. Indices were derived from continuous, discrete and mixed characters. **A**, cultivated putative hybrids; **B**, field-grown putative hybrids.

Figure 4.25. Frequency distributions of range-standardised characters for *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species. **A,** Discrete characters; **B,** continuous characters (rounded to one decimal place and grouped into classes with 0.1 intervals).


A
cumulative frequency
40
35
30
25
20
15
10
5
0
E. sinclairii 1
E. sinclairii 2
E. sinclairii 3
E. sinclairii 4
E. sinclairii 5
F1
F2
F9
F10
F11
F13
S1
S2
S3
A. bellidioides 1
A. bellidioides 2
A. bellidioides 3
A. bellidioides 4
A. bellidioides 5
A. bellidioides 6
1
0.5
0
B
cumulative frequency
18
16
14
12
10
8
6
4
2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
character value
A. bellidioides
E. sinclairii
F1
F2
F9
F10
F11
F13
S1
S2
S3

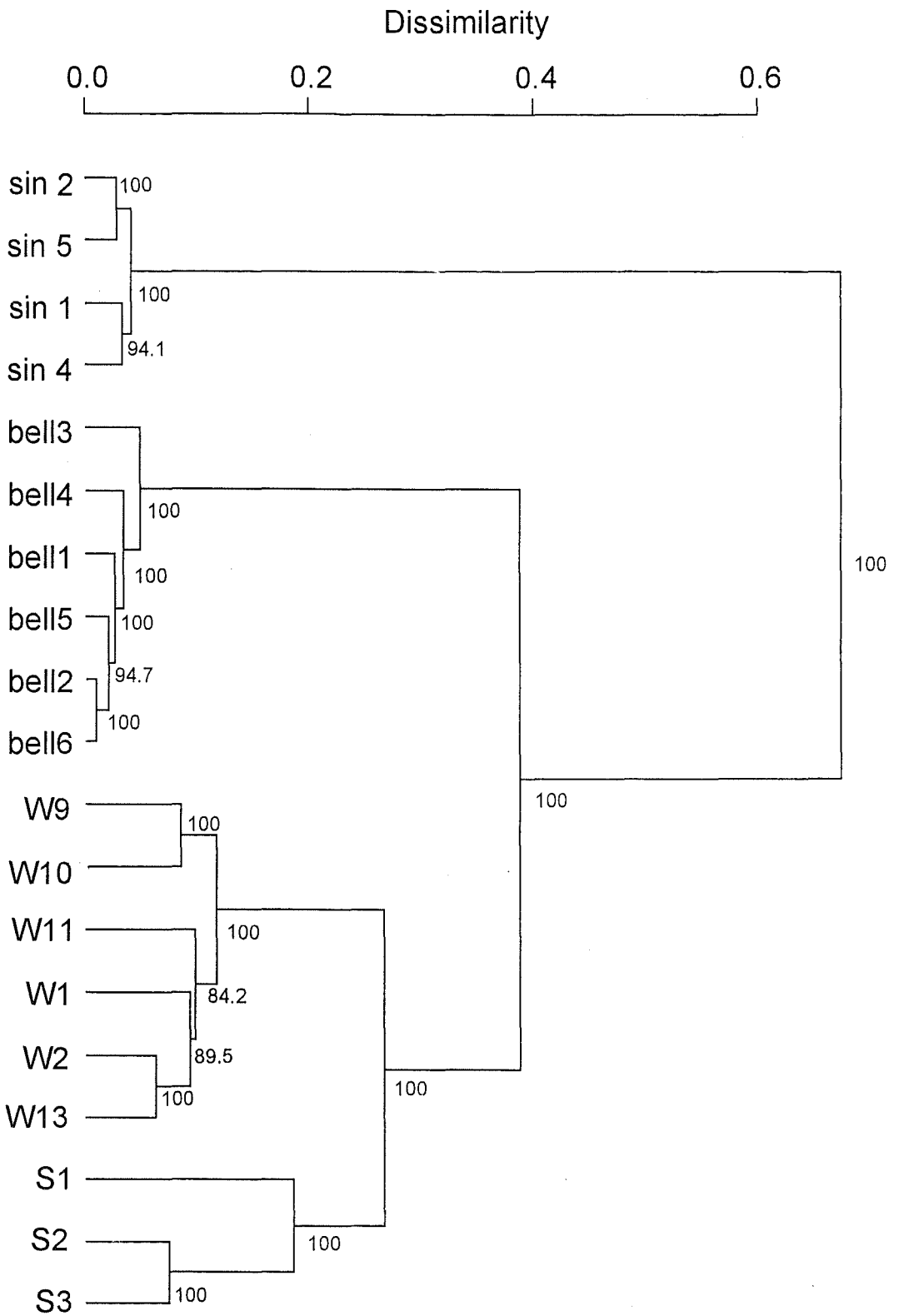


Figure 4.26. Phenogram generated by agglomerative hierarchical clustering, with group-average linkage, of dissimilarities between *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species. Percentage support values from an OTU-based jackknife analysis are given for each junction.

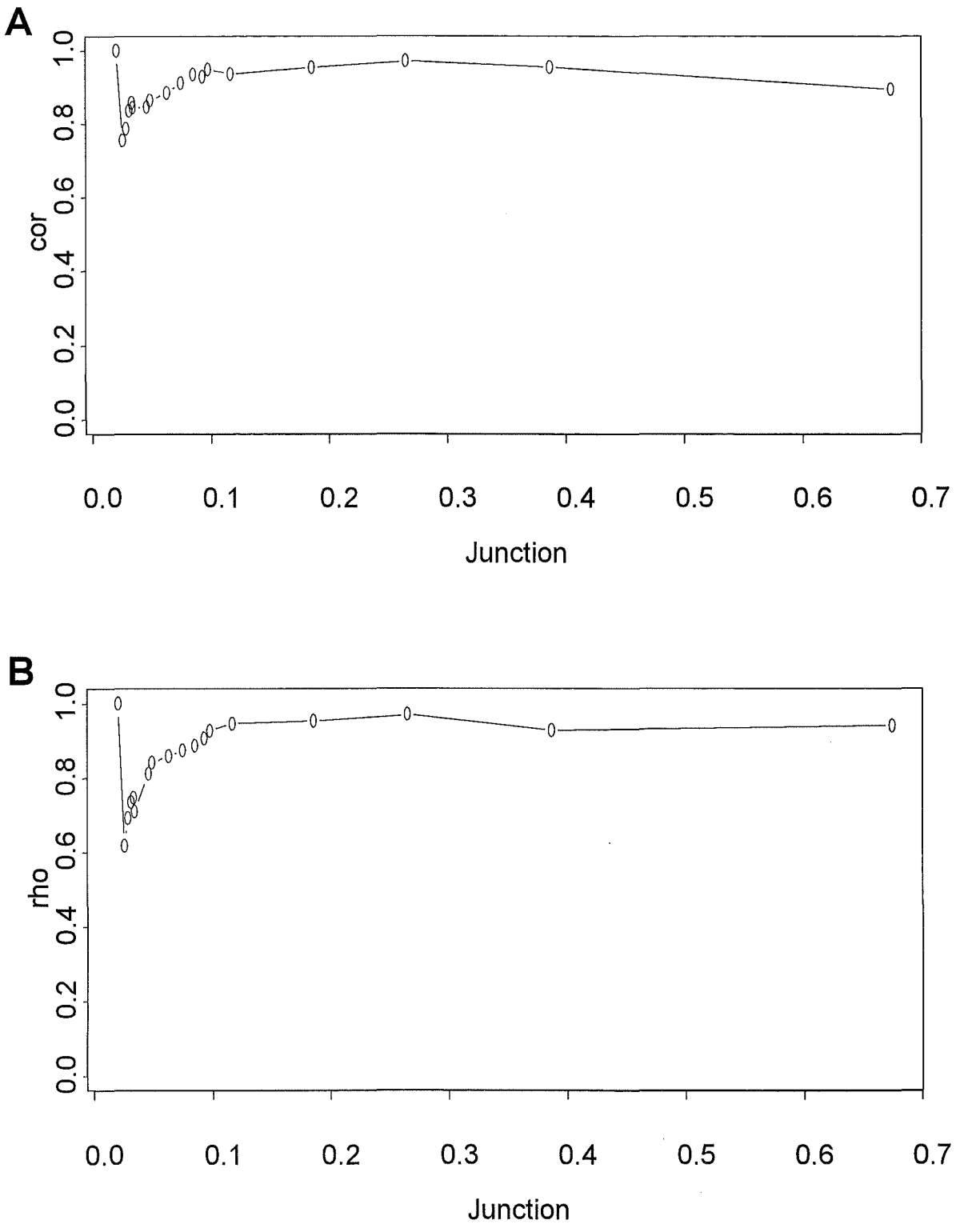


Figure 4.27. Correlation coefficients for each junction of the phenogram presented in Figure 4.26. **A,** Pearson's product-moment correlation coefficient; **B,** Spearman's rank correlation coefficient.

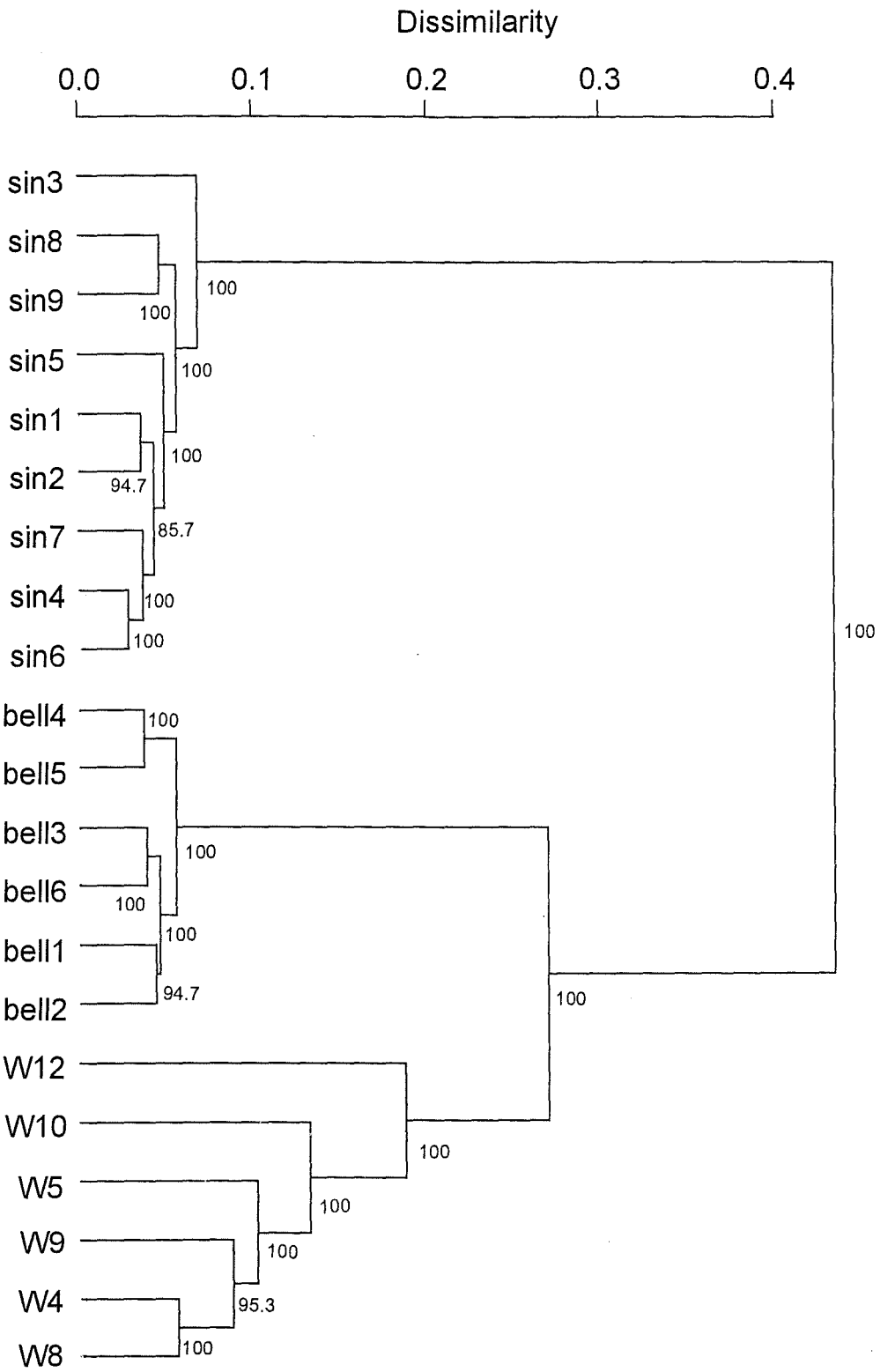


Figure 4.28. Phenogram generated by agglomerative hierarchical clustering, with group-average linkage, of dissimilarities between *Anaphalioides bellidioides*, *Ewartia sinclairii* and field-grown putative hybrids between the two species. Percentage support values from an OTU-based jackknife analysis are given for each junction.

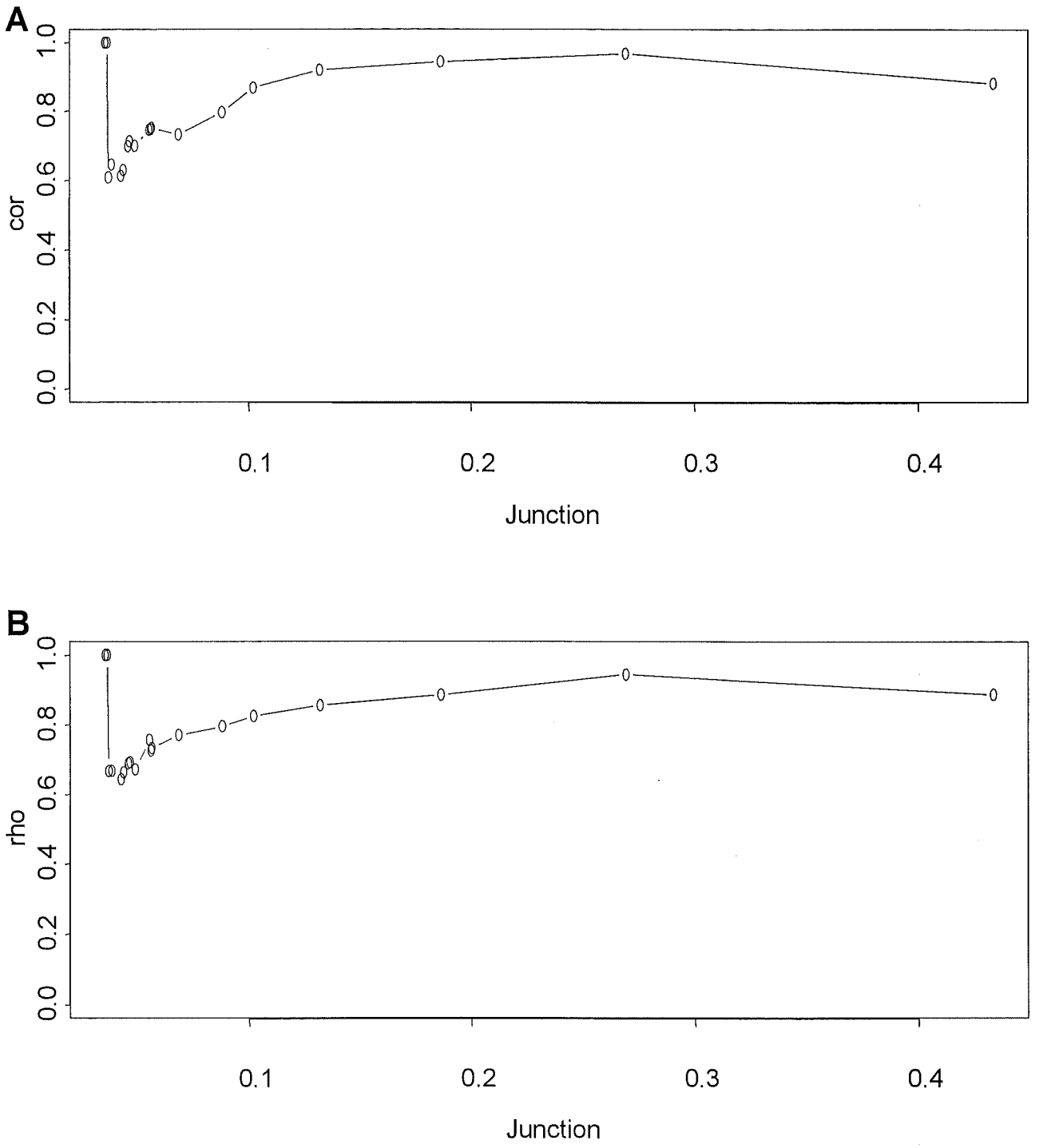


Figure 4.29. Correlation coefficients for each junction of the phenogram presented in Figure 4.28. **A,** Pearson's product-moment correlation coefficient; **B,** Spearman's rank correlation coefficient.

Weights	*W1*		*W2*		*W9*		*W10*		*W11*		*W13*		*S1*		*S2*		*S3*		*A. bellidioides* individuals		*E. sinclairii* individuals	
	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F
Continuous characters																						
wI=1, wE=1, wP=1	29	8	18	13	15	41	3	81	11	57	27	1	0	0	0	0	11	25	0	0	0	0
wI=0.1, wE=1, wP=1	26	5	18	23	17	27	2	104	24	4	27	1	0	0	0	0	0	0	0	0	0	0
wI=1, wE=0.1, wP=1	24	12	13	6	11	39	3	73	0	0	19	2	15	19	5	18	24	1	0	0	0	0
wI=1, wE=1, wP=0.1	29	9	18	7	13	23	4	92	10	37	28	1	0	0	0	0	12	24	0	0	0	0
wI=0.1, wE=0.1, wP=1	16	1	14	4	13	6	11	7	15	5	17	2	9	8	5	16	14	3	0	0	0	0
wI=1, wE=0.1, wP=0.1	25	22	13	1	9	17	1	110	0	0	18	3	20	49	5	32	23	9	0	0	0	0
Discrete characters																						
wI=1, wE=1, wP=1	30	1	26	50	0	0	0	0	30	8	28	34	0	0	0	0	0	0	0	0	0	0
Mixed characters																						
wI=1, wE=1, wP=1	30	1	24	37	0	0	2	112	30	58	28	6	0	0	0	0	0	0	0	0	0	0
wI=1, wE=1, wP=1†	NA	NA	NA	NA	30	15	30	1	NA	NA	NA	NA	0	0	2	86	25	61	0	0	0	0
wI=1, wE=0.1, wP=1	27	1	21	2	20	27	13	58	15	70	18	8	0	0	0	0	0	0	0	0	0	0
wI=0.1, wE=0.1, wP=1	26	1	17	6	9	26	12	18	24	9	25	2	0	0	0	0	1	92	0	0	0	0
wI=1, wE=1, wP=0.1	30	1	24	36	0	0	0	0	30	55	30	5	0	0	0	0	0	0	0	0	0	0

Table 4.13. Summary of HYWIN analyses comparing *A. bellidioides*, *E. sinclairii* and cultivated putative hybrids between the two species. The highest-ranking combinations as indicated by the 0.95 probability level were evaluated in each analysis. Weights: wI = intermediacy; wE = equality; wP = parental distance. †, reanalysed after exclusion of the four highest-ranking putative hybrids. N = number of times ranked as a hybrid; F = rank of first time as a hybrid; 0 = never ranked; NA = excluded from the analysis.

HYBRID	PARENT 1	PARENT 2	RANK	IN	EQ	PD	NP	HS
S3	bell281/4	sin140/1	1	0.638	-0.167	0.851	0.354	1.5723
W13	bell281/5	sin145	2	0.666	-0.016	0.802	0.394	1.5658
W13	bell281/5	sin140/5	3	0.680	0.009	0.782	0.388	1.5609
S3	bell281/4	sin140/2	4	0.644	-0.147	0.831	0.354	1.5601
S3	bell281/4	sin145	5	0.649	-0.139	0.823	0.354	1.5584
W2	bell139/1	sin145	6	0.694	-0.119	0.774	0.341	1.5561
W13	bell139/1	sin145	7	0.682	-0.052	0.774	0.367	1.5515
W2	bell139/1	sin140/5	8	0.705	-0.095	0.754	0.341	1.5494
S3	bell281/4	sin140/5	9	0.650	-0.118	0.803	0.354	1.5413
W13	bell139/2	sin140/5	10	0.663	0.002	0.776	0.388	1.5392
W13	bell139/2	sin145	11	0.644	-0.024	0.796	0.389	1.5382
W1	bell281/4	sin140/1	12	0.593	-0.062	0.851	0.408	1.5377
W13	bell139/1	sin140/5	13	0.682	-0.027	0.754	0.367	1.5338
W13	bell281/4	sin145	14	0.605	0.010	0.823	0.408	1.5269
W13	bell281/5	sin140/1	15	0.598	-0.053	0.830	0.394	1.5232
W13	bell281/4	sin140/5	16	0.622	0.035	0.803	0.388	1.5213
W2	bell281/5	sin140/5	17	0.638	-0.057	0.782	0.369	1.5146
S2	bell281/4	sin140/1	18	0.596	-0.343	0.851	0.283	1.5130
S1	bell281/4	sin140/1	19	0.584	-0.237	0.851	0.329	1.5113
W2	bell281/5	sin145	20	0.616	-0.080	0.802	0.369	1.5100
W1	bell281/4	sin145	21	0.584	-0.030	0.823	0.408	1.5036
S2	bell281/4	sin145	22	0.611	-0.320	0.823	0.283	1.5024
W1	bell139/1	sin140/1	23	0.609	-0.096	0.803	0.381	1.5014
W2	bell139/1	sin140/1	24	0.613	-0.150	0.803	0.341	1.5008
W2	bell139/2	sin140/5	25	0.630	-0.064	0.776	0.363	1.5001
W1	bell281/4	sin140/5	26	0.596	-0.007	0.803	0.408	1.4989
W13	bell139/1	sin140/1	27	0.604	-0.089	0.803	0.367	1.4981
W1	bell281/5	sin140/1	28	0.574	-0.062	0.830	0.408	1.4980
W2	bell139/2	sin145	29	0.609	-0.088	0.796	0.363	1.4962
W13	bell139/2	sin140/1	30	0.576	-0.060	0.824	0.389	1.4947
S2	bell281/4	sin140/2	31	0.595	-0.327	0.831	0.283	1.4934
S1	bell281/4	sin140/2	32	0.584	-0.218	0.831	0.329	1.4931
W1	bell139/2	sin140/1	33	0.576	-0.075	0.824	0.398	1.4929
W2	bell281/4	sin140/5	34	0.592	-0.029	0.803	0.390	1.4923
W1	bell281/4	sin140/2	35	0.563	-0.040	0.831	0.408	1.4905
S3	bell281/4	sin140/3	36	0.633	-0.143	0.772	0.354	1.4902
W13	bell281/4	sin140/1	37	0.541	-0.026	0.851	0.416	1.4897
W2	bell281/4	sin145	38	0.572	-0.052	0.823	0.390	1.4894
W9	bell139/1	sin145	39	0.624	-0.106	0.774	0.357	1.4875
S3	bell139/2	sin140/1	40	0.580	-0.191	0.824	0.338	1.4849
W1	bell281/3	sin140/1	41	0.567	-0.088	0.822	0.388	1.4797
S1	bell281/5	sin140/1	42	0.574	-0.253	0.830	0.318	1.4789
S3	bell139/2	sin145	43	0.596	-0.163	0.796	0.338	1.4758
S3	bell281/5	sin140/1	44	0.562	-0.166	0.830	0.356	1.4750
S3	bell281/3	sin140/1	45	0.570	-0.205	0.822	0.328	1.4709
S2	bell281/4	sin140/5	46	0.598	-0.304	0.803	0.283	1.4705
S1	bell281/4	sin145	47	0.568	-0.211	0.823	0.329	1.4703
W2	bell281/5	sin140/1	48	0.551	-0.111	0.830	0.369	1.4700
S1	bell281/4	sin140/5	49	0.585	-0.191	0.803	0.329	1.4688
W9	bell139/1	sin140/5	50	0.622	-0.083	0.754	0.357	1.4683

Table 4.14. Results of a HYWIN analysis of 16 continuous characters comparing *A. bellidioides*, *E. sinclairii* and cultivated putative hybrids using the following weights: wI = 0.1, wE = 0.1, wP = 1. Only the 50 highest-ranking combinations are listed. IN = intermediacy score; EQ = equality score; PD = parental distance score; NP = distance to the nearest parent; HS = hybrid optimality score. bell = *A. bellidioides*; sin = *E. sinclairii*.

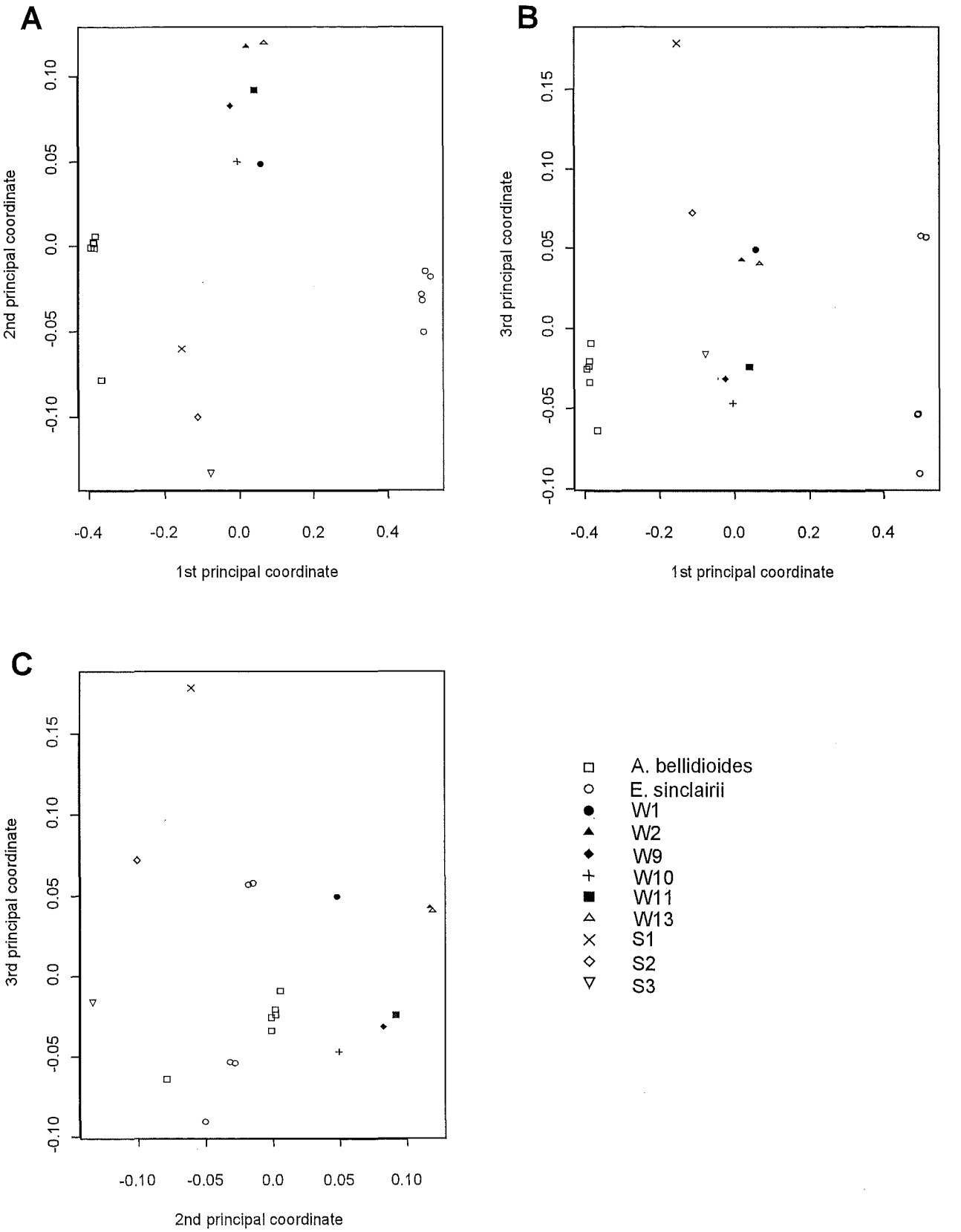


Figure 4.30. Scatter plots of the first, second and third principal coordinates for *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species. **A**, First versus second principal coordinate; **B**, first versus third principal coordinate; **C**, second versus third principal coordinate.

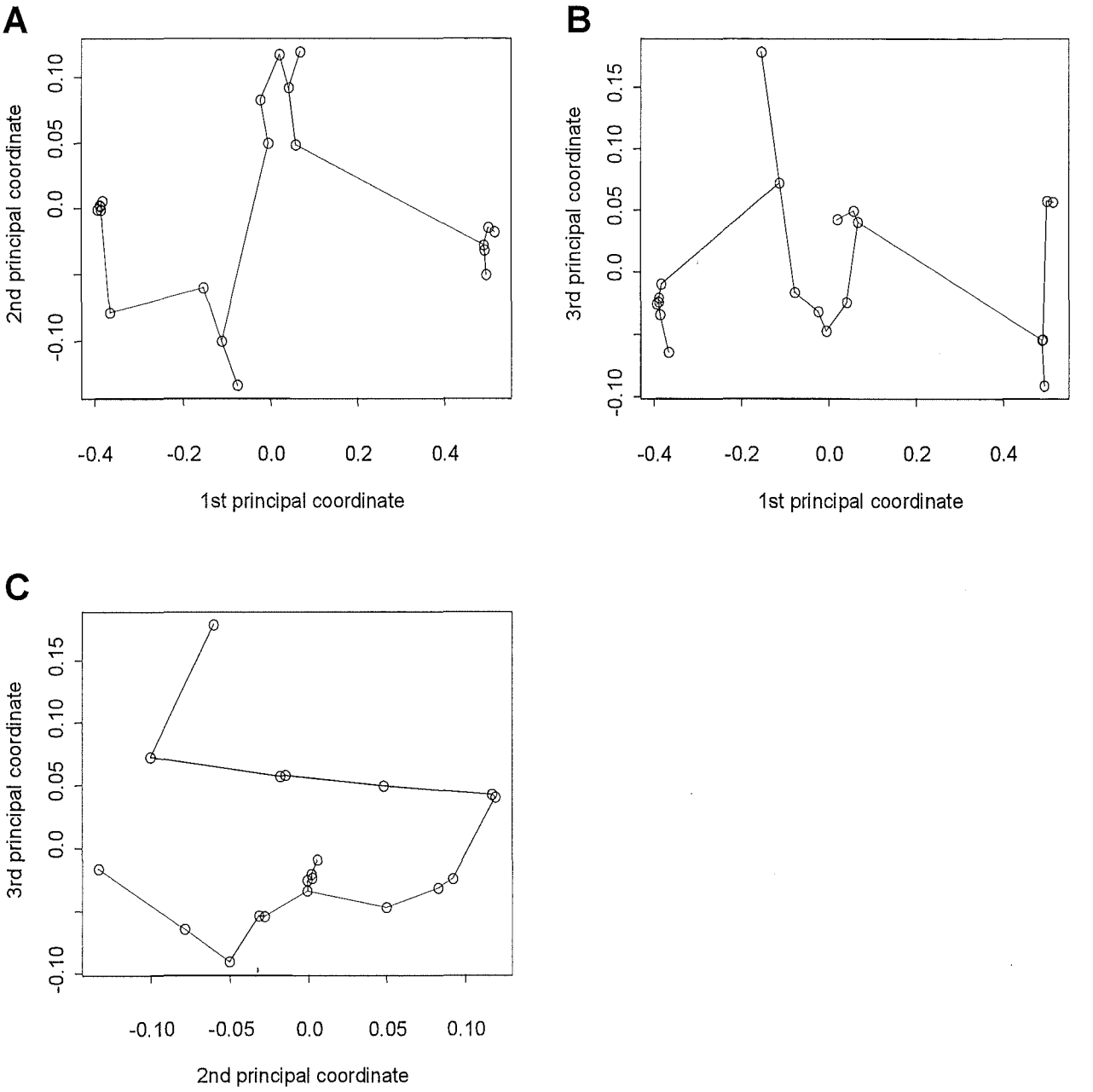


Figure 4.31. Scatter plots of the first, second and third principal coordinates (as presented in Figure 4.30) with a minimal spanning tree constructed on each plot.

Figure 4.32. Splits graphs derived from mixed characters for *Anaphalioides bellidioides*, *Ewartia sinclairii* and cultivated putative hybrids between the two species.

A, Split decomposition (drawn to scale). Fit = 76.8 %.

B, Split decomposition (drawn with equal edges). Fit = 76.8 %.

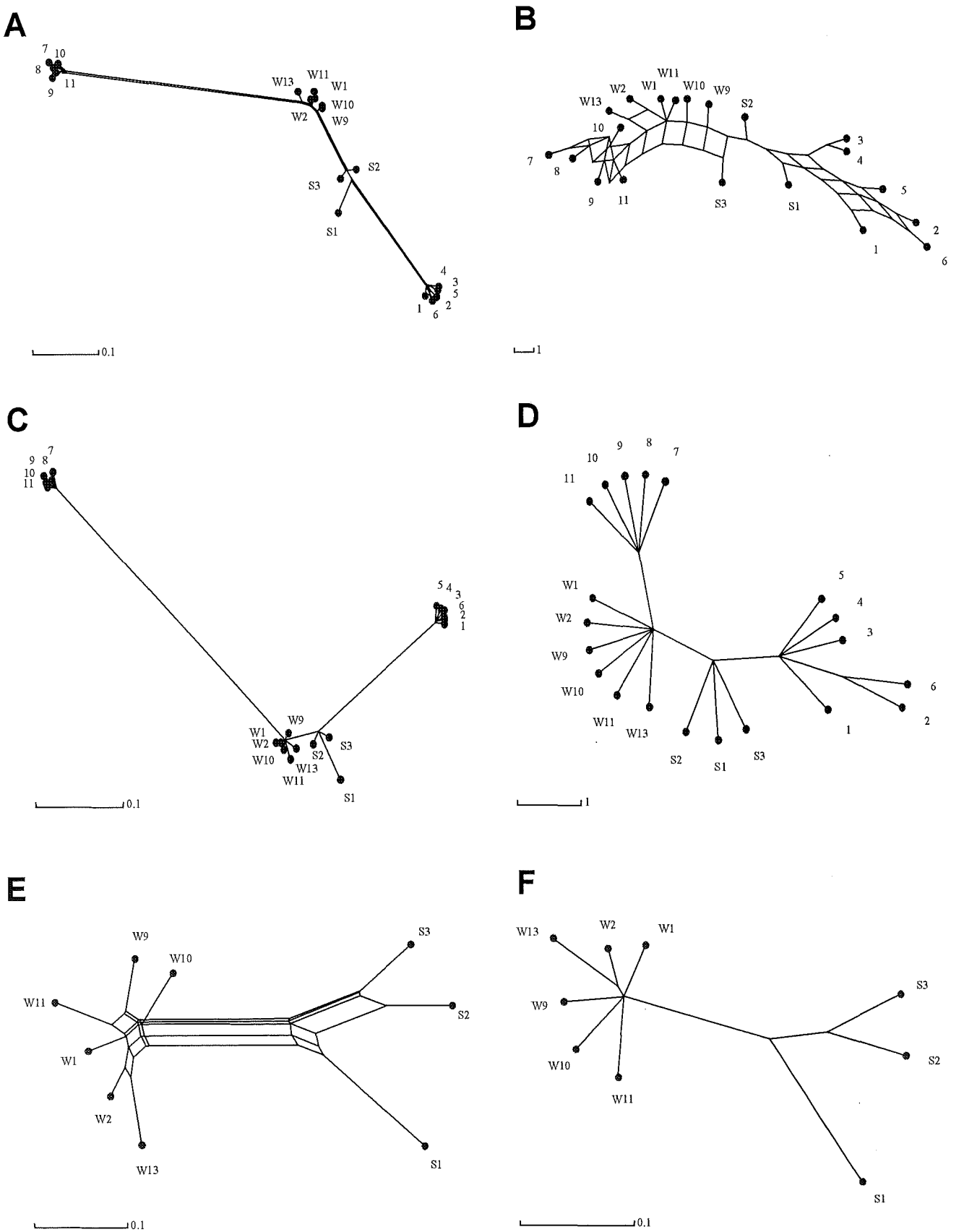
C, Buneman tree (drawn to scale). Fit = 61.6 %.

D, Buneman tree (drawn with equal edges). Fit = 61.6 %.

E, Split decomposition following exclusion of the putative parental species (drawn to scale). Fit = 98.2 %.

F, Buneman tree following exclusion of the putative parental species (drawn with equal edges). Fit = 74.6 %.

Key to the species: 1–6, *A. bellidioides;* 7–11, *E. sinclairii.*


A
7
10
8
11
9
W13
W11
W1
W10
W2
W9
S2
S3
S1
4
3
5
1
6
2
0.1
B
W11
W2
W1
W10
W9
W13
S2
10
7
8
3
4
9
11
S3
S1
5
2
1
6
1
C
7
9
8
10
11
5
4
3
6
2
1
W9
W1
W2
W10
S3
S2
W13
W11
S1
0.1
D
9
8
10
7
11
5
W1
4
W2
3
W9
6
W10
2
W11
W13
1
S2
S1
S3
1
E
W9
S3
W10
W11
S2
W1
W2
W13
S1
0.1
F
W13
W2
W1
S3
W9
S2
W10
W11
S1
0.1

4.3.5 Experimental crosses among *A. bellidioides*, *E. sinclairii* and the putative hybrids

Crosses between *A. bellidioides* and *E. sinclairii*

All individuals of *A. bellidioides* and *E. sinclairii* tested and all of the putative hybrids were strongly self-incompatible (see pp. 294–295). Reciprocal crosses between single plants of *E. sinclairii* and *A. bellidioides* collected from the study site was successful in both directions (Table 4.15 p. 175). When *E. sinclairii* was the maternal parent, 89 % of the cypselas matured, but only 19 % matured when *A. bellidioides* was the maternal parent. Aborted cypselas were rare; three cypselas (from a total of 74 florets pollinated) had enlarged but were shrivelled and empty in the cross *E. sinclairii* × *A. bellidioides*, but all cypselas were either filled or unenlarged in the reciprocal crosses. The cross *E. sinclairii* × *A. bellidioides* yielded the highest proportion of mature cypselas of any cross performed in this thesis between individuals of *A. bellidioides*, *E. sinclairii* and the putative hybrids. In addition, the reciprocal difference in the cross-compatibility of these two individuals was the most marked of any cross between individuals of *A. bellidioides*, *E. sinclairii* and the putative hybrids.

Crosses between the putative parental species and putative hybrids

Fourteen crosses between putative hybrids and individuals of *A. bellidioides* and *E. sinclairii* were performed. Mature cypselas were produced in all but two of the crosses; only reciprocal crosses between *W13* and a plant of *E. sinclairii* failed to yield mature cypselas. Aborted cypselas were rare (in most crosses comprising less than 5 % of the total cypsela number), but in three crosses – *W2* × *E. sinclairii*, *W2* × *W13* and *W13* × *A. bellidioides* – aborted cypselas made up 45 %, 42 % and 16 % respectively of the total cypsela number.

Field-collected putative hybrids were successfully crossed with both *A. bellidioides* and *E. sinclairii*. Mature cypselas were obtained from the crosses *E. sinclairii* × *W9* (40 %) and *A. bellidioides* × *W9* (12 %). Reciprocal crosses between *W13* and a plant of *A. bellidioides* yielded 12 % and 33 % mature cypselas. In contrast, as mentioned above, reciprocal crosses between *W13* and a plant of *E. sinclairii* were unsuccessful in both directions and no cypselas had enlarged. In all other reciprocal crosses performed between *A. bellidioides*, *E. sinclairii* and the putative hybrids, mature cypselas were obtained in both directions.

The seed-raised putative hybrid *S2* was successfully crossed with two individuals of *A. bellidioides* (yielding 1-31 % mature cypselas in reciprocal crosses) and with one plant of *E.*

Maternal parent	Paternal parent	Date	Capitula pollinated	Florets pollinated	Estimate of style retraction (%)	Total cypselas	Mature cypselas	Aborted cypselas	Unenlarged cypselas
A. bellidioides I/3	*E. sinclairii* B	14/10/96	3	all female	ND	357	69	0	288
A. bellidioides I/3	*W9*	19/10/97	3	all female	25	307	38	0	269
A. bellidioides I/3	*W13*	19/10/97	2	all female	25	215	59	1	155
A. bellidioides I/3	*S1*	22/10/97	1	all female	0	94	2	0	92
A. bellidioides I/3	*S2*	22/10/97	3	all female	0	283	2	0	281
E. sinclairii B	*A. bellidioides* I/3	14/10/97	9	all female	90	74	66	3	5
E. sinclairii B	*W9*	14/10/97	16	all female	75	120	48	1	71
E. sinclairii B	*W13*	14/10/97	11	all female	ND	110	0	0	110
E. sinclairii B	*S2*	22/10/97	14	all female	25	92	11	4	77
W2	*A. bellidioides* H	22/10/96	3	all female	50	110	8	0	102
W2	*E. sinclairii* C	22/10/96, 25/10/96	5	all female	75	33	10	15	8
W2	*W13*	14/10/96, 15/10/96,	2	all female	10	89	9	37	43
W11	*W13*	29/9/97	8	all female	ND	377	19	0	358
W13	*A. bellidioides* I/3	19/10/97	3	all female	90	99	33	16	50
W13	*E. sinclairii* B	19/10/97	8	all female	0	243	0	0	243
W13	*W11*	22/9/97, 27/9/97	7	all female	90	220	32	2	186
W13	*S2*	30/10/97	4	all female	75	139	0	0	139
S2	*A. bellidioides* I/1	30/10/97	2	all female	10	105	33	1	71
S2	*A. bellidioides* I/3	22/10/97	3	all female	0	152	28	0	124
S2	*E. sinclairii* B	22/10/97	3	all female	75	170	106	1	63
S2	*W9*	22/10/97	3	all female	75	197	104	27	66

Table 4.15. Results of experimental crosses between individuals of *Anaphalioides bellidioides*, *Ewartia sinclairii* and natural putative hybrids between the two species. ND, data not collected. For a key to plant provenances, see Appendix 2.

sinclairii (12 % and 62 % seed set in reciprocal crosses). In these crosses, mature cypselas were more frequent when *S2* was the maternal parent. A cross between *S1* and a plant of *A. bellidioides* yielded 2 % filled cypselas. Mature cypselas were also obtained when *W2* was pollinated by a plant of *A. bellidioides* collected from Ashley Gorge, Canterbury and a seed-raised plant of *E. sinclairii* from the Hodder valley, Inland Kaikoura Range, Marlborough.

Crosses between putative hybrids

Three crosses – *W2* × *W13*, *W13* × *W11* and *W11* × *W13* – were performed between field-collected putative hybrids. Each of these crosses yielded mature cypselas but seed set was low (10 %, 15 % and 5 % respectively). In the cross *W2* × *W13* 42 % of the cypselas had aborted, but in reciprocal crosses between *W11* and *W13* aborted cypselas comprised less than 1 % of the total cypsela number. *S2* was successfully crossed with *W9*, yielding 53 % mature cypselas. No cypselas enlarged in the cross *W13* × *S2*, one of only three crosses in which no mature or aborted cypselas were produced. However, 75 % of the styles were estimated to have retracted in this cross.

Germination of seed from experimental crosses

Mature cypselas from 12 experimental crosses were sown on 20 April 1998. Over 80 % of the seeds germinated for seven crosses and about 50 % germinated for four crosses (Table 4.16 p. 186). The lowest germination rate (27.4 %) was obtained for seeds from a cross between *S2* and a plant of *E. sinclairii.* In each of two crosses (*A. bellidioides* × *S1* and *A. bellidioides* × *S2*) only two mature cypselas were obtained, but the seeds of all four germinated readily (after 12–40 days). The seeds from crosses between *A. bellidioides* and putative hybrids germinated relatively quickly (a minimum of 9 days and only four seeds took 70 days or more to germinate), but seeds from all crosses involving plants of *E. sinclairii* were notably slower to germinate, often taking over 100 days to germinate. Seeds from a reciprocal cross between plants of *A. bellidioides* and *E. sinclairii* were relatively slow to germinate but every seed germinated. An extremely wide range of germination times was notable for some crosses (e.g., *S2* × *W9*).

cross	number sown	number germinated	germination (%)	range (d)	mean	s.d.	median	First quartile	Third quartile
A. bellidioides B × *E. sinclairii* B	69	69	100	13–133	100.7	16.5	99	93	110
A. bellidioides B × *W9*	38	34	89.5	9–116	36.3	26.4	28	18	49.5
A. bellidioides B × *W13*	33	32	97	9–73	30.3	18.8	25.5	17	38
A. bellidioides B × *S1*	2	2	100	23–40	31.5	12	31.5	27.3	35.8
A. bellidioides B × *S2*	2	2	100	12–13	12.5	0.7	12.5	12.3	12.8
E. sinclairii B × *A. bellidioides* B	66	66	100	44–152	94.6	27.8	92	70	108.5
E. sinclairii B × *W9*	48	22	45.8	38–134	104.5	26.9	113.5	104.5	122.3
W11 × *W13*	19	10	52.6	12–116	49.1	31.1	43.5	30	63.8
W13 × *A. bellidioides* B	33	16	48.5	9–33	17.5	7.7	16	11	19
S2 × *A. bellidioides* B	28	24	85.7	9–57	20.2	12	16	12	26.3
S2 × *E. sinclairii* B	106	29	27.4	107–157	143.4	13.7	149	138	152
S2 × *W9*	104	50	48.1	9–155	55.5	42.4	37	22	72.8

Table 4.16. Germination of seed from experimental crosses involving plants of *A. bellidioides*, *E. sinclairii* and putative hybrids between the two species. For a key to plant provenances, see Appendix 2.

4.3.6 Records of other wild hybrids

CHR 8660 and 333738

A single plant (Herbarium L. Cockayne 4626; CHR 8660, 333738) was collected from Robinson Creek in the upper Awatere valley by Leonard Cockayne and Charles Foweraker in January 1912. The plant was described as growing on rock at about 1000 m (Cockayne, 1916). Duplicate sheets of this collection exist (CHR 8660, 333738). CHR 8660 is the type specimen for *Helichrysum fowerakeri*. On the label for CHR 333738 Cockayne had added in pencil, "now published by me as a hybrid. *H. bellidioides* × *Sinclairii*".

CHR 8660 comprises two flowering shoots, but the lefthand specimen is typical *E. sinclairii*. The shoot from the putative hybrid bears two solitary capitula. CHR 333738 comprises three shoots: one bearing six capitula, one with a solitary capitulum and in the other the capitulum has been lost or detached. Cockayne's Latin diagnosis for *H. fowerakeri* (Cockayne, 1916) and the overall morphology of the type specimen suggests a close affinity with the Yeo Stream putative hybrids, highlighted by the following characters: production of solitary capitula and multicapitulate inflorescences; presence of bracteate leaves on the flowering shoots; leaf shape and possession of a short mucro (upturned on some leaves); density of the leaf indumentum; and inner involucral bracts with a white, radiating lamina 3–3.5 mm long.

capitula and multicapitulate inflorescences; presence of bracteate leaves on the flowering shoots; leaf shape and possession of a short mucro (upturned on some leaves); density of the leaf indumentum; and inner involucral bracts with a white, radiating lamina 3–3.5 mm long. Pappus-hair morphology and the presence of ovary trichomes in the type specimen were not investigated.

CHR 8661

This specimen was collected by Leonard Cockayne and is labelled as *Helichrysum fowerakeri*, but no other collection details are recorded on the herbarium label and I am unaware of any published reference to this specimen. The sheet contains two stems, one of which bears six multicapitulate inflorescences.

Its morphology differs markedly from all other putative *A. bellidioides* × *E. sinclairii* hybrids examined in this thesis. The leaf was somewhat spathulate in shape with a distinct, narrow petiole present. The lamina was clearly green but the petiole and often the lamina margins are chocolate-brown. A distinct mucro, plane with the lamina, was present. The lamina tip is usually rounded and the lamina margins are shallowly undulate. The midrib was raised on the abaxial surface and often depressed on the adaxial surface. The capitula were small (about 2 mm × 4 mm) with very short peduncles 1–2 mm long and were borne in dense, almost spherical clusters. The lamina of the inner involucral bracts was short (1–2 mm long), often slightly straw coloured and the tip was acute to obtuse. The involucral bract gap was distinctly pinkish. Four hermaphrodite florets were examined. Twin hairs (similar in size and structure to those of putative *A. bellidioides* × *E. sinclairii* hybrids) were present on the ovary. The pappus hairs had one to three, clavate, protruding apical cells with uniformly thickened cell walls. Red-purple pigmentation was present in the upper corolla tube. Most of these features are suggestive of *Helichrysum lanceolatum* and *A. bellidioides* × *H. lanceolatum* hybrids (Jordan, 1995; and my own observations) and no characters unique to *E. sinclairii* were identified. Thus its morphology suggests it is not a hybrid between *A. bellidioides* and *E. sinclairii*, but rather might be an *A. bellidioides* × *H. lanceolatum* hybrid.

CHR 18210

This plant was collected from the Awatere valley by George Simpson in March 1937. The sheet contains a single large shoot with more than 20 lateral shoots bearing multicapitulate inflorescences. The morphology of the leaves, capitula, involucral bracts and pappus hairs

indicates a close similarity with the Yeo Stream putative hybrids. In particular, leaf shape and dimensions (6–12 mm × 2–3 mm), the presence of a distinct mucro, which was upturned in some leaves, inner involucral bracts with a white, radiating lamina 2.5–3 mm long, and the morphology of the pappus-hair apical cells were similar. No discordant characters were observed.

CHR 82713

Only the collector (George Simpson) is specified on the herbarium label for this specimen. The sheet contains three flowering shoots. The largest stem has about 20 lateral shoots bearing inflorescences of one to seven capitula. The leaves were somewhat smaller (5–8 mm × 1.5–3 mm) in this plant and the leaf tip obtuse, but other leaf characters, capitulum and pappus-hair morphology, and the absence of ovary twin hairs (in two female florets and three hermaphrodite florets examined) were similar to CHR 18210 and the Yeo Stream putative hybrids. No discordant characters were observed.

CHR 82714

This specimen was collected from the upper Dee River in February 1938. The sheet contains two large flowering shoots and three small shoots. The overall leaf, capitulum and pappus-hair morphology is very similar to the Yeo Stream putative hybrids. The leaves are moderate in size (6–11 mm × 2–3.5 mm) and have a short, often upturned mucro. Most inflorescences are multicapitulate with up to six capitula. Five glabrous ovaries (floret types unknown owing to abscission of the corolla tube) were observed. No discordant characters were observed.

CHR 87682

Allan (1961) suggested this specimen might be a hybrid between *A. bellidioides* and *E. sinclairii*. George Simpson collected it from the Dee River, but the herbarium label lacks any other collection details. The sheet contains two large stems bearing one to seven capitula per flowering shoot. The leaves were 5–7 mm × 2–3 mm with a very short, often upturned mucro. The overall leaf and capitulum morphology is very similar to the Yeo Stream putative hybrids. No discordant characters were observed.

CHR 385817

A single putative hybrid (CHR 385817) was collected from the upper Hodder River by Brian Molloy on 19 February 1981 and subsequently cultivated at the DSIR Botany Division (now

Landcare Research), Lincoln. FAA-preserved flowering shoots from the cultivated plant, a herbarium voucher and photographs of the cultivated plant in flower remain from this collection. The herbarium specimen comprises two large stems, each bearing a single pedunculate capitulum. On the label the plant is recorded as growing on a bank in tussock grassland at about 4800 ft (1463 m).

The growth habit of CHR 385817 was clearly intermediate between *A. bellidioides* and *E. sinclairii* (Plate 7 A–C p. 182) and similar to that of the Yeo Stream putative hybrids. Its floral morphology was also extremely similar to that of the Yeo Stream putative hybrids (Figure 4.33 A–L p. 183). One to five pedunculate capitula were present on each flowering shoot. The inner involucral bracts had white hygroscopic laminae and the stereome had narrow hyaline margins and dense indumentum on the abaxial surface. The floret ratios, pappus hair morphology and the dimensions of the capitulum, receptacle, involucral bracts, corolla tubes and pappus hairs were very similar to those of the Yeo Stream putative hybrids. However, the corolla lobes of hermaphrodite florets were usually erect. The presence of sparse twin hairs on the ovary of female florets was another distinctive character shared with certain Yeo Stream putative hybrids, but in CHR 385817 biseriate glandular trichomes were also occasionally present – the largest observed was 150 μm in length and comprised seven cells. A subconical and scrobiculate receptacle were two other distinctive characters shared by CHR 385817 and certain Yeo Stream putative hybrids. Leaves from the base of the preserved flowering stems of CHR 385817 were similar in size, shape, leaf apex shape, mucro angle, indumentum density and venation to comparable leaves from field-grown Yeo Stream putative hybrids. The presence of type B glandular trichomes on the leaves and the structure of the type A trichomes is further evidence of an affinity with the Yeo Stream putative hybrids.

The morphological evidence thus suggests a close affinity between CHR 385817 and the Yeo Stream putative hybrids. The greatest differences observed in CHR 385817 were the presence of occasional glandular trichomes on the ovary of female florets and the usually erect corolla lobes of hermaphrodite florets.

CHR 385826

This specimen was also collected on 19 February 1981 by Brian Molloy from the upper Hodder River. On the herbarium label, Molloy determined this plant to be a trispecific cross between *Helichrysum coralloides* and *A. bellidioides* × *E. sinclairii*. However, Williams

(1989), in a list of Gnaphalieae hybrids recorded by Molloy from the Hodder valley, lists *Helichrysum depressum* × "*H. fowerakeri*". The herbarium sheet contains two sterile shoots. The label records the plant as growing on riverbed gravels at about 4800 ft (1463 m) with the three putative parental species growing nearby. The sheet also bears a handwritten remark in pencil, "No evidence of *H. bellidioides* here (sterile) D. Glenny 21/5/96".

The trinervate leaves are tightly appressed to the stem and have cucullate tips lacking a distinct mucro. Dense indumentum covers the adaxial surface and the lower abaxial surface, with type B-like clothing trichomes present in the distal portion of the adaxial surface (a characteristic of *H. coralloides*). Clothing trichomes are absent in the distal portion of the abaxial surface. Thus the leaf morphology is typical of a whipcord *Helichrysum* and no evidence for *A. bellidioides* or *E. sinclairii* was observed in its vegetative morphology. In the absence of capitula it is difficult to determine its identity conclusively from morphological evidence alone, but it seems more likely the plant is a *H. coralloides* × *H. depressum* hybrid.

4.4 Discussion

Evidence for hybridity

Pollen stainability and artificial crosses provided evidence for reduced fertility among the cultivated putative hybrids, and meiotic abnormalities in microsporocytes were observed in *W9*, all of which are consistent with the hypothesis that the plants were of hybrid origin. The rarity of extreme and novel morphological characters and predominance of intermediacy among continuous characters in the putative hybrids provided further support for the hybridity hypothesis. The following morphological characters, in particular, provided strong evidence for the putative hybrids' parentage: the length of the white, hygroscopic lamina of the inner involucral bracts; the colour of the corolla lobes, anthers and pollen; the presence and density of twin hairs on the cypselas of the female florets, and their absence on hermaphrodite florets; and the morphology of the pappus hair tips. *Anaphalioides bellidioides* was strongly implicated as one parent, but evidence for the second parental species was less conclusive based on field-grown specimens. Although some continuous characters suggested *H. coralloides*, *H. parvifolium* or *O. leptophyllus* as possible parents, discrete characters provided strong evidence that *E. sinclairii* was the other parent. Flowering of *Anaphalioides bellidioides* and *Ewartia sinclairii* was coincident at the study site and in the glasshouse, and germination of seeds from artificial crosses provided evidence that production of viable hybrids between the two species was possible.

Plate 7. A putative hybrid between *Anaphalioides bellidioides* and *Ewartia sinclairii* (CHR 385817) collected from the upper Hodder valley, Inland Kaikoura Range by Brian Molloy. Photographed in cultivation at DSIR Botany Division, Lincoln, 1981.

A, *E. sinclairii* (left), CHR 385817 (centre), *A. bellidioides* (right) (photo Brian Molloy).

B, CHR 385817 (photo Brian Molloy).

C, CHR 385817 (photo Brian Molloy).


A


B
5
4
3
2
1
0
cm


C

Figure 4.33. Morphological features of a putative hybrid between *Anaphalioides bellidioides* and *Ewartia sinclairii* (CHR 385817) collected from the upper Hodder valley, Inland Kaikoura Range by Brian Molloy.

A, Venation of a leaf from the base of a flowering shoot. Scale = 2 mm.

B, Type A glandular trichome from abaxial leaf surface. Scale = 20 μm.

C, Type B glandular trichome from leaf margin. Scale = 20 μm.

D, Female floret. Scale = 1 mm.

E, Hermaphrodite floret. Scale = 1 mm.

F, Tip of a pappus hair from a female floret. Scale = 100 μm.

G, Tip of a pappus hair from a hermaphrodite floret. Scale = 100 μm.

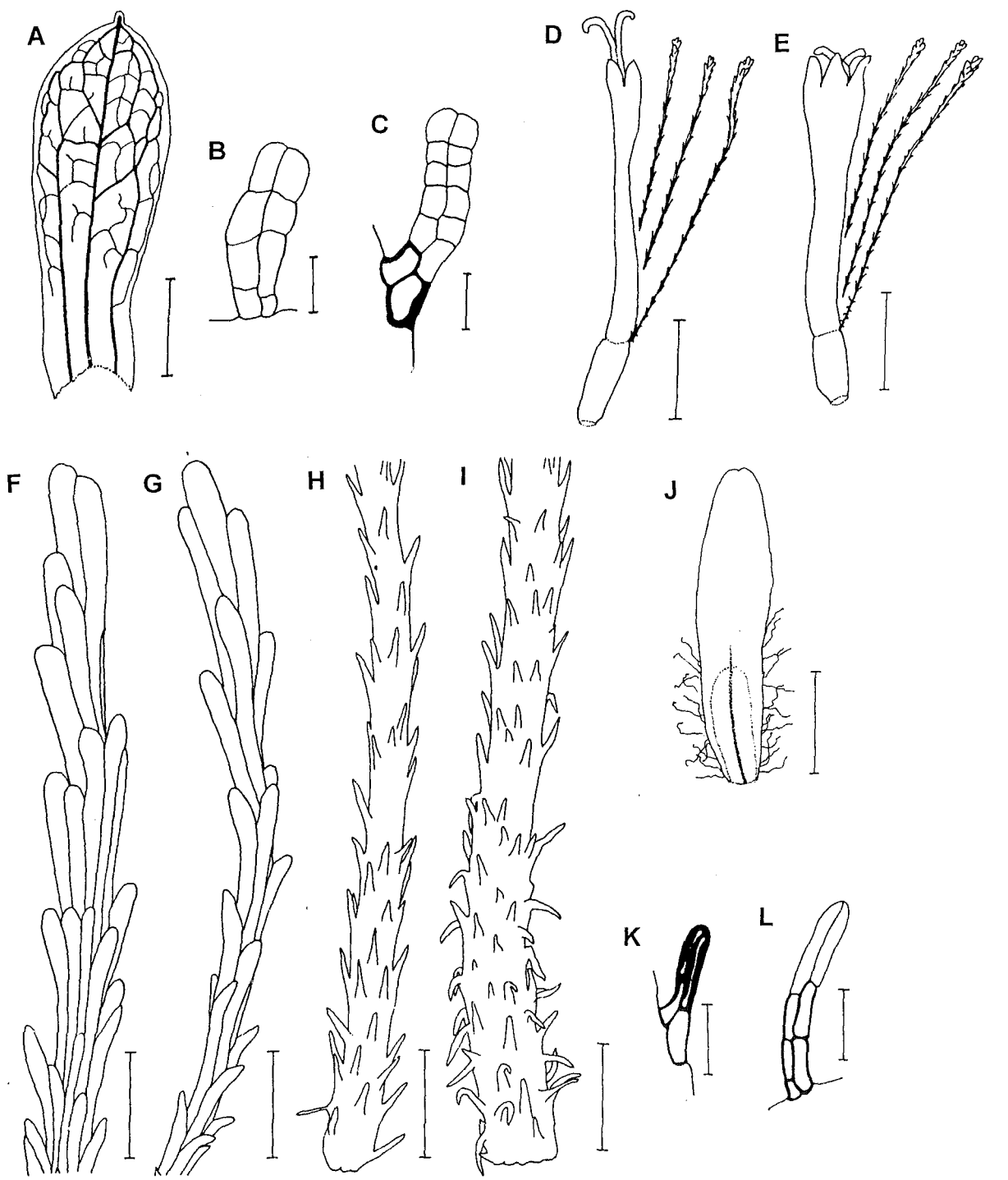
H, Base of a pappus hair from a female floret. Scale = 100 μm.

I, Base of a pappus hair from a hermaphrodite floret. Scale = 100 μm.

J, Inner involucral bract. Scale = 2 mm.

K, Twin hair from ovary of a female floret. Scale = 50 μm.

L, Multicellular twin hair from ovary of a female floret. Scale = 50 μm.


A
B
C
D
E
F
G
H
I
J
K
L

Comparison of the data analyses for cultivated and field-grown specimens indicated continuous characters for the field-grown plants were less reliable than for cultivated plants. For example, evidence that *W10* was a hybrid between *A. bellidioides* and *E. sinclairii* was weak based on continuous data from field-grown shoots, but the plant was intermediate between the putative parental species based on data from cultivated clones. Sample size might have been a factor in certain instances, as data for some putative hybrids were recorded from a single capitulum. The morphology of *W12* (particularly continuous characters) could have been dramatically affected by the health of the rootstock or the plant channelling all its resources into the sole live shoot. The field-collected specimens of the putative hybrids were collected in different years (1989 and 1998) and would have been subjected to differing environmental influences. Although data from cultivated plants were, overall, concluded to be of higher predictive value, continuous leaf characters were still notably variable. The utility of molecular genetic data for detecting hybridity and characterising hybrids is well established (see Chapter 7) and it is likely techniques such as isozyme analysis and identification of microsatellite markers would allow more reliable determination of the putative hybrids between *A. bellidioides* and *E. sinclairii*.

Characterisation of the putative hybrids from morphology

Most analytic methods consistently discriminated two groups (the seed-raised and field-collected plants) among the cultivated putative hybrids. The seed-raised putative hybrids were usually intermediate between *A. bellidioides* and the other putative hybrids, but in some characters clustered with the field-collected putative hybrids. Most methods also indicated *S1* was dissimilar to the other two seed-raised putative hybrids. The field-grown putative hybrids were generally represented as a single, more variable group. The morphology of the pappus hair tips and the presence or absence of ovary twin hairs demonstrated that *W1* and *W2* (collected by Josephine Ward and John Lovis in 1989) were different plants to those collected in 1995 and 1998.

A priori synthesis of artificial hybrids to provide information on the genetic regulation of morphological characters in hybrids allows identification of hybrids from morphology with greater confidence (Baker, 1947) and the contribution of genetic effects such as dominance, matrocliny and heterosis can be assessed. Although morphology provided strong evidence for parentage in the present study, in the absence of artificial hybrids it is difficult to characterise the hybrids as F_1, later-generation or backcross hybrids. Parental and intermediate character states were present in similar proportions in *W1*, *W2*, *W4*, *W5*, *W8*, *W11* and *W13*. However, it

is unknown whether this is what would be predicted for F_1 hybrids. Segregation would be expected to increase the frequency of parental and extreme character states relative to intermediate characters and result in increased variation in the complement of parental characters in at least some hybrids. In *W9* and *W10*, *A. bellidioides* characters exceeded *E. sinclairii* characters by approximately two to one, which could indicate they are later-generation hybrids or backcrosses with *A. bellidioides*. Most analytic methods placed *W9* and *W10* slightly closer to *A. bellidioides* than the other field-collected putative hybrids. The non-intermediacy of field-grown clones of *W9* and *W10* on some canonical variates and principal coordinates probably reflects environment-induced variation of continuous characters in field-grown specimens, as cultivated clones were consistently intermediate between the putative parental species. Different analytic methods suggested conflicting affinities for *W4*, *W5*, *W8* and *W12*, which also probably reflected environmental effects on continuous characters from field-grown specimens.

Several findings could indicate most of the field-collected plants were F_1 hybrids. Overall, there was limited morphological variation among the field-collected putative hybrids and extreme and novel characters were rare. Only leaf dimensions varied notably among the putative hybrids. Thus either segregation in later generations has not contributed to their genetic makeup or, alternatively, certain genotypes have greater fitness at the site. Artificial crosses suggested F_1 hybrids were the most readily synthesised and that strong barriers to the production of backcross and later-generation hybrids exist. The probability that all extant hybrids differed from those collected in November 1989 (J. D. Lovis, pers. comm.) means the extant hybrids must be of recent origin.

Canonical discriminant analysis, cluster analysis and MDS indicated *W12* was the most dissimilar of the field-grown putative hybrids, but there was little suggestion of it belonging to a distinct group and its affinities with other putative hybrids varied with analytic method. Certain continuous floral characters (corolla tube and pappus hair lengths in both floret types) exhibited a parental character state in *W12*, but were intermediate in other field-collected putative hybrids. Whether this reflects genetic differences, environmental influences or plant health is uncertain. *W12* was difficult to classify because some data were unavailable and, as already mentioned, continuous characters may have been influenced by the plant's health or the plant channelling all its resources into the single live shoot. Character indices highlighted the disparity in expression of discrete and continuous characters in this plant.

S1

The maternal parent of *S1* was the putative hybrid *W12*. Since all other putative hybrids and individuals of *A. bellidioides* and *E. sinclairii* tested were highly self-incompatible, the likelihood is low that *S1* resulted from self-fertilisation of *W12*. The identity of the paternal parent is inconclusive from morphological evidence, made more difficult by the inability to propagate *W12* and thus compare continuous characters. In all analyses *S1* was slightly separated from the other putative hybrids, largely owing to its possession of two novel characters, but was closest to *S2* and *S3*. Given the preponderance of *A. bellidioides* characters over *E. sinclairii* characters, *S1* could be a backcross to *A. bellidioides*, but an equally possible hypothesis is that *W9* was the paternal parent. The two novel characters recorded for *S1* suggested *O. leptophyllus* might be the second parent, but other morphological evidence strongly discounted this possibility and no flowering or fruiting *O. leptophyllus* plants were observed in the Yeo valley at the time of collection of *W12*.

S2 and *S3*

The morphology of *S2* and *S3* was very similar, the two plants differing principally in corolla tube length, pappus hair length, the presence of twin hairs on the ovary of female florets and the shape of the involucral-bract lamina tip. In many discrete characters *S2* and *S3* were identical to *A. bellidioides* and they differed from the other putative hybrids in several characters, such as indumentum density on the adaxial leaf surface and stomata level. The discrete characters not shared with *A. bellidioides* (the possession of recurved corolla lobes, narrow hyaline margins on the bract claws, reddish pigmentation in the bract gap, the type of wall thickening in the pappus hair tips, the bract tip shape in *S3* and the presence of occasional twin hairs on the ovary of female florets in *S2*) all suggested the contribution of *E. sinclairii* to their genomes. In most continuous characters the two plants clustered with or were intermediate between the other putative hybrids and *A. bellidioides*. Each plant possessed two extreme characters and neither plant possessed novel characters. All analytic methods suggested they were most similar to *S1* and slightly closer to *A. bellidioides* than the other field-collected putative hybrids. When the seed was collected, plants of *E. sinclairii* and the putative hybrids *W9* and *W12* were also in flower, any of which could be the paternal parents of *S2* and *S3*. The preponderance of *A. bellidioides* characters could reflect maternal dominance effects in an F_1 hybrid, but it seems more likely that *W9* or *W12* was the paternal parent and thus *S2* and *S3* were backcross hybrids. Their strong superficial resemblance to *A. bellidioides* suggests that backcrosses might be easily overlooked in the field.

Putative hybrids from other localities

Morphological evidence suggested six plants collected from four other localities in Marlborough are of the same origin as the Yeo Stream putative hybrids. All of the localities lie within the geographical range of *E. sinclairii*. Other than the original collection (CHR 8660, 333738), for which only a single plant was discovered (Cockayne, 1916), collectors gave no indication of the frequency of the putative hybrids at the other localities. The high similarity between the Yeo Stream putative hybrids and CHR 8660 confirms that the validly published name *Helichrysum fowerakeri* Cockayne applies to hybrids between *A. bellidioides* and *E. sinclairii*, but because they are intergeneric hybrids publication of a nothogeneric name is desirable to maintain taxonomic order.

Natural hybrids involving *E. sinclairii* appear to be rare. Other than the hybrids with *A. bellidioides*, only one putative hybrid with *Helichrysum lanceolatum* photographed by S. Courtnay (photograph seen) is recorded. A putative hybrid with a *Raoulia* species collected from "Mt Schiza" by William Martin (WELT 78632) was examined but determined to be a specimen of *Raoulia bryoides* Hook.f. The specimen is near barren but a single female floret and involucral bract were examined and no evidence for *E. sinclairii* was observed. Sympatry, flowering phenology and pollinator preferences might limit the opportunities for *E. sinclairii* to hybridise in the field. In this thesis a plant of *E. sinclairii* was cross-compatible with *Euchiton audax* and *E. traversii* (see Chapter 6) and single filled cypselas were obtained in crosses with *Helichrysum intermedium* and *Ozothamnus leptophyllus*, but germination of the seeds is needed to confirm their parentage. In contrast, *A. bellidioides* is comparatively promiscuous and is cross-compatible with many indigenous Gnaphalieae, both in the field and in artificial crosses (see Chapters 2 and 6).

Character expression

The characters included in an investigation of hybridity can strongly influence the conclusions reached. In this case study, character counts, character indices and scatter plots of continuous characters demonstrated how very different conclusions could be drawn from different characters or character sets, justifying the inclusion of a wide range of characters. Unfortunately, experimental hybrids could not be studied within the time limitations of this thesis, so the influence of dominance, heterosis or matrocliny is unknown. The frequencies of extreme, intermediate, novel and parental characters are likely to differ between F_1, later-generation and backcross hybrids, but might be unpredictable even in artificial hybrids due to the genotype of the parents.

In character counts most discrete characters were classified as parental in the putative hybrids, but this partially reflects the difficulty of coding some discrete characters to allow identification of an intermediate state. Some continuous characters were variable between putative hybrids (e.g., leaf dimensions, receptacle dimensions and corolla-tube length), but capitulum dimensions and involucral-bract dimensions were homogeneous. Continuous floral characters generally exhibited less within-plant variation than leaf dimensions and so demonstrated the intermediacy of the putative hybrids more clearly.

Leaf dimensions of cultivated plants exhibited notable within-plant variation, despite efforts to minimise variation induced by environmental conditions and developmental stage. However, differences *between* putative hybrids indicated the existence of genetic differences. The leaf anatomy of the cultivated putative hybrids was intermediate in most characters discriminating the putative parental species, with stomata level being notably variable. Lamina shape and leaf apex shape were other variable vegetative characters. Discrete floral characters varying among the putative hybrids included: involucral bract tip shape, reddish pigmentation in the bract gap, apical cells distinctly protruding in pappus hairs of female florets, and crimson pigmentation in the corolla lobes.

Only two novel characters (multicellular twin hairs on the ovary of female florets in *S1* and CHR 385817, and occasional twin hairs on the ovary of hermaphrodite florets in *S1*) were recorded in the putative hybrids. Ovary trichomes might be readily mutable or these characters might be present in the parental populations but absent in the sampled plants. Multicellular twin hairs were more frequent in CHR 385817 and so may be more common in either or both parental populations in the upper Hodder valley. Phenotypic characters under simple genetic control are likely to be more mutable than those under polygenic control. Novel and extreme characters are not uncommon in hybrids, especially among later-generation hybrids (Rieseberg and Ellstrand, 1993). In the Compositae novel characters have been reported, for example, in F_1 hybrids between *Matricaria recutita* and *Tripleurospermum tetragonaspermum* (Mitsuoka and Ehrendorfer, 1972) and F_4 hybrids between *Helianthus annuus* and *Verbesina helianthoides* (Vassilevska-Ivanova *et al.*, 1996).

Pollen potential

It was concluded that the putative hybrids had reduced pollen potential, but further experimentation (such as germination tests) is required to gain a more accurate estimate of pollen fertility. However, no one method currently exists to measure pollen viability or

fertility reliably for all plants (Dafni and Firmage, 2000) and *in vitro* germination of Compositae pollen is difficult (e.g., Hoekstra and Bruinsma, 1975). Alexander's differential stain and the fluoroscein diacetate reaction (FCR) provided evidence for reduced pollen fertility in the cultivated putative hybrids, but both methods have limitations (see p. 340). Trinucleate pollen, as possessed by the Compositae, has short viability (Brewbaker, 1967), at least under humid conditions (Hoekstra and Bruinsma, 1975), but only freshly presented pollen was stained in this thesis. Thus the low FCR reaction in *W11* could not have resulted from loss of viability between pollen presentation and testing. The reduced reaction by *A. trivervis* pollen indicated FCR is sensitive to pollen quality and might have underestimated the pollen potential of *W11*. Hiscock (2000a) also reported a low proportion (16.5–53 %) of fluorescent pollen grains with the FCR reaction in *Senecio squalidus*. Some grains that would be classified as abnormal with Alexander's stain produced a positive FCR reaction, but such grains may still lack the potential to achieve *in vivo* germination and fertilisation, as in some Compositae respiration in pollen grains continues for a considerable period after the ability to germinate has been lost (Hoekstra and Bruinsma, 1975).

Unusually large and tetraporate (rather than triporate) pollen grains have been observed in artificial hybrids in the Compositae (Crisp and Jones, 1978; Kyhos *et al.*, 1990), a condition associated with unreduced ploidy in the Compositae (Barrier *et al.*, 1999). No such abnormal pollen grains were observed in the putative hybrids between *A. bellidioides* and *E. sinclairii*, but the presence of small pollen grains with little or no stainable cytoplasm appeared to be related to the production of micronuclei during meiosis.

Factors influencing hybridisation in the field

Artificial crosses demonstrated the cross-compatibility of *A. bellidioides*, *E. sinclairii* and some of the putative hybrids, the potential for formation of backcross and later-generation hybrids, and high seed viability in many of the crosses performed. However, natural hybrids are known from only five localities and a total of 17 field-collected hybrids, 12 of which are from the Yeo valley, together with the three seed-raised hybrids studied in this thesis. The raising of *S1*, *S2* and *S3* provided evidence that viable backcross and later-generation hybrids are produced in the field. The experimental crosses suggested that production of F_1 hybrids is easier than formation of backcrosses and later generations, but additional crosses are required to confirm this and the strong reciprocal difference in cross-compatibility between plants of *A. bellidioides* and *E. sinclairii* still represents a major barrier to hybridisation. The artificial

crosses are likely to overestimate cross-compatibility in the field, e.g. due to lower pollen loads and the possible effects of pollen competition.

Ewartia sinclairii is widespread in the Awatere and Clarence drainage areas but *A. bellidioides* appears to be limited by moisture availability, hence the species are not commonly sympatric. The severity of the climate and the instability of the substrate would restrict the availability of favourable habitats and hinder establishment of hybrid seedlings. Hybrids often occur in disturbed habitats, where competition from other plants is reduced (Focke, 1881 p. 494). There is ample evidence for instability and natural disturbance along much of the Yeo valley and the slip to the left of the study site was not present in 1989 (J. D Lovis, pers. comm.), indicating the instability of the riverbank at the study site.

Experimental crosses indicated various post-pollination barriers to hybridisation exist. The unsuccessful reciprocal cross between a plant of *E. sinclairii* and *W13* might be due to the possession of identical self-incompatibility (*S*) alleles by both plants, and shared *S* alleles might have prevented some pollen grains from germinating on the stigma in other crosses. The self-incompatibility system is likely to be a greater barrier to the formation of backcross and later-generation hybrids, especially between siblings, and might also contribute to reciprocal differences in cross-compatibility. Plants with sporophytic self-incompatibility (such as the Compositae) often yield reciprocal differences in cross-compatibility when the two parents share one *S* allele (Richards, 1997). Reduced gamete viability and reduced vigour of viable gametes might also have contributed to the low seed set in most crosses involving putative hybrids. The occurrence of enlarged but empty and shrivelled cypselas in some crosses suggested post-zygotic abortion of the embryo or endosperm had occurred, but in most crosses they were rare or absent. It is possible early zygote abortion before the cypsela had visibly enlarged was more common, but estimates of style retraction generally agreed with the proportion of enlarged cypselas. A notable exception was the cross *W13* × *S2*, in which an estimated 75 % of the styles retracted but no cypselas enlarged. Failure of pollen tubes to fertilise ovules or early zygoye abortion may have occurred in this cross.

Pollen competition might be an important factor in the field. Conspecific pollen might outcompete heterospecific pollen in the style, restricting production of F_1 hybrids, and pollen from either species might outcompete hybrid pollen, restricting the production of backcross hybrids. Pollen competition is one explanation for the frequency of F_1 hybrids in artificial crosses in *Haplopappus* (Smith, 1968; Smith, 1970) and *Helianthus* (Heiser *et al.*, 1969;

Rieseberg *et al.*, 1995) and is considered important in Louisiana iris crosses, particularly in restricting formation of the F_1 generation (Arnold, 1997 pp. 90-98).

Reduced fitness of hybrids, relative to that of the parental species, might also limit the frequency of hybrids between *A. bellidioides* and *E. sinclairii*. Seven seeds from the capitulum of *W12* germinated, but only one plant (*S1*) survived to maturity, suggesting at least some hybrid genotypes are weak. Later-generation hybrids, in particular, may have reduced fitness owing to genic disharmony and splitting of coadapted gene complexes. The survival to maturity of particular phenotypes may be favoured in the field. Studies on other plants and animals demonstrate that fitness can vary between different hybrid genotypes and with different fitness measures (Arnold, 1997 pp. 140-143; Arnold and Hodges, 1995). Alternatively, one or both parental species might have migrated to the site only recently and so the hybrids are an indicator of only recent reproductive interaction between the two species. The probable recent origin of the extant putative hybrids might have allowed little time for later-generation hybrids and backcrosses to become established at the study site. A further possibility is that most hybrids are short-lived in the field. The largest plant at the site in December 1995 (*W9*) was 40 cm in diameter, whereas the other putative hybrids were either immature or in poor health.

The capitula of the New Zealand Gnaphalieae seem ideally adapted to the New Zealand pollinating fauna (Wilton, 1997 p. 107), which lacks specialised pollinators. The capitula lack adaptations for wind pollination, such as pendant capitula and increased pollen per floret, present in some Compositae (e.g., Payne, 1963; Garnock-Jones, 1986; Berry and Calvo, 1989). A variety of generalist insect visitors are recorded on indigenous Gnaphalieae (Primack, 1983; Wilton, 1997 pp. 196-199). Visitors observed on *A. bellidioides* capitula by these authors were the moth *Dasyuris anceps* Butler, Coleoptera, Syrphidae (hoverflies), Tachinidae and other unidentified Diptera. Insect visitors to *E. sinclairii* capitula in the field have not been investigated. Floral visitors to either species were not monitored at the study site, but a single copper butterfly (*Lyceana sallustius* Fabriccus) was observed on *A. bellidioides* capitula at the study site in December 1998. Monitoring of floral visitors in the field is required to determine the degree of overlap and frequency of visitors between the two species, which may be factors limiting hybridisation.

Some factors are likely to favour hybridisation between the two species. The two species and the putative hybrids had coincident flowering periods at the Yeo Stream site (as observed in

the 1995-96 and 1998-99 growing seasons) and in the glasshouse. The close proximity of plants of each species and the sparse vegetation at the study site (rendering capitula of the two species more prominent and attractive to floral visitors) may also enhance the opportunity for hybridisation. The lower number of *A. bellidioides* plants relative to *E. sinclairii* may be a significant factor. Focke (1881 p. 463) noted that natural hybrids are more likely to occur when plants of one parental species are rare and grow intermingled with a more common relative, especially if the uncommon species is self-sterile. Situations in which one species is in full flower and only the first or last flowers of a related species are open are also conducive for hybridisation (Focke, 1881 pp. 463–464). All of the putative hybrids and individuals of both species tested appeared to be highly self-incompatible. However, additional sampling is required to determine the breeding system of the parental species populations.

In conclusion, morphology and field evidence strongly supported the hybridity hypothesis. Pollen stainability and experimental crosses (indicating reduced fertility in the putative hybrids) and the occurrence of meiotic abnormalities in *W9* were consistent with a hybrid origin. Artificial crosses demonstrated the cross-compatibility of *A. bellidioides* and *E. sinclairii*. The seed-raised putative hybrids were clearly discriminated from the field-collected putative hybrids on morphology. *S2* and *S3* appeared to be backcross hybrids between *A. bellidioides* and a putative hybrid; *S1* might be either a backcross or later-generation hybrid. Characterisation of the field-collected putative hybrids was inconclusive. The partial fertility of the putative hybrids, and the raising of the seed-raised hybrids, indicates the potential for backcross and later-generation hybrids to arise. Six putative hybrids from four other localities in Marlborough were morphologically comparable to the Yeo Stream plants. The rarity of hybrids between *A. bellidioides* and *E. sinclairii* in the field could reflect a variety of factors: a low frequency of hybridisation, owing to pollinator differences, pollen competition and limited frequency of sympatry; internal post-pollination barriers; substrate instability and limited availability of suitable habitats; and low hybrid fitness.

Chapter 5. Case study 2: *Leucogenes grandiceps* (Hook.f.) Beauverd × *Raoulia eximia* Hook.f.

5.1 Introduction

The South Island edelweiss (*Leucogenes grandiceps*) occurs over much of the South Island from northwest Nelson to Southland, but in the northeast it is absent north of the Awatere River and only rarely occurs east of the Wairau River (Molloy, 1995). It is also found on Stewart Island. It inhabits rock outcrops, ledges and stable debris in fellfield. *Raoulia eximia* is one of the species colloquially known as 'vegetable sheep'. It inhabits rock outcrops and fellfield in the South Island and occurs predominantly east of the main divide as far south as northern Otago. The two species occur at subalpine and alpine altitudes and are commonly sympatric in Canterbury. Putative hybrids between the two species have been collected from a number of localities in central and southern Canterbury. Molloy (1980) reported *L. grandiceps* × *R. eximia* occurring on Coal Hill, Tara Haoa Range and on Tripps Peak, Four Peaks Range. Other collectors have discovered putative hybrids on Mt Peel, Mt Potts, Mt Torlesse and the Ohau Ski Basin (see pp. 253–255). For the present study putative hybrids were collected from Mt Hutt, Mt Hutt Range, southern Canterbury.

The objectives of this case study were identical to those outlined in Chapter 4. The primary aim was to determine the identity of the putative hybrids from morphology and leaf anatomy. Additional objectives were to gain information on character expression, fertility and meiotic pairing in the putative hybrids and possible hybridisation barriers between the putative parental species, and to compare relationships among the putative hybrids and sympatric species using several multivariate analytic methods.

5.2 Materials and methods

Unless stated otherwise, the methodology was identical to that employed in Chapter 4.

5.2.1 The study site

The study site was visited during three growing seasons (1996-97, 1998-99 and 2000-01). The site was heterogeneous with areas of herbfield, tussock grassland, fellfield and rock outcrops. Prevalent plants included *Anisotome aromatica* Hook.f., *Celmisia* species (particularly *C. angustifolia* Cockayne, *C. lyallii* Hook.f. and *C. spectabilis* Hook.f.), *Chionochloa macra* Zotov, *Dracophyllum pronum* W.R.B.Oliver, *Gaultheria depressa*

Hook.f., *Kelleria dieffenbachii* (Hook.) Endl., *Luzula traversii* (Buchanan) Cheeseman and *Poa* L. species. Seven Gnaphalieae species were located in the vicinity of the putative hybrids. *Anaphalioides bellidioides* was most common growing in open stony situations, on stable scree margins and beside rock outcrops forming mats up to 60 cm across. *Leucogenes grandiceps* was common on rock outcrops and in open rocky situations and usually formed small, open patches. *Raoulia eximia* was common on rock outcrops and and formed cushions up to 1.3 m across. *R. mammillaris* grew in identical situations but was less common and formed smaller cushions up to 30 cm in diameter. *R. grandiflora* and *R. subsericea* were common in open rocky situations and among other low-growing herbs and shrubs.

Putative hybrids between *L. grandiceps* and *R. eximia* were scattered at the site, growing in open stony situations or on rock outcrops. Specimens from eleven putative hybrids were collected for study (Table 5.1 p. 195). The majority were small (less than 5 cm in diameter) immature plants, but five plants greater than 5 cm in diameter were located and capitula collected from four of these plants. *W1–W6* were immature plants or seedlings less than 5 cm in diameter and not in flower when specimens were collected in January 1997. *W7* was 10 cm in diameter and bore six capitula in January 1996. *W8* was a semi-cushion plant 20 cm in diameter but suffering about 40 % dieback. This plant flowered in January 1999 but not in January 2001. *W9* (10 cm in diameter) and *W10* (5 cm in diameter) were smaller plants with a semi-cushion growth habit. *W9* flowered during January 1999 and both *W9* and *W10* flowered during January 2001. *W11* was 10 cm in diameter and formed a more upright cushion, but was never seen in flower. A further putative hybrid (*W12*), collected from Mt Hutt and cultivated by Joan Whillans, was also included in the study. *W13* was a putative hybrid between *L. grandiceps* and *R. mammillaris* collected from the study site; no capitula from this plant were available for study.

5.2.2 Plant specimens available for study

Specimens from up to ten individual plants of each gnaphalioid species growing at the study site were collected at random. Each species was morphologically well differentiated and 'pure' individuals were readily identifiable. Only field-collected capitula from four putative hybrids between *L. grandiceps* and *R. eximia* were available for study, as flowering of these plants in cultivation has never been recorded and did not occur during the present study. Therefore field-collected capitula were used for description of floral characters from all plants studied. Vegetative characters were described from clones cultivated in an unheated glasshouse in order to minimise environment-induced variation. Two attempts to grow cuttings from *W9*

Identification number in this thesis	Identification number or herbarium voucher number	Specimens from cultivated plants	Capitula from field-growing plants
Putative *Leucogenes grandiceps* × *Raoulia eximia*:			
W1	*R.J.McKenzie 189*	+	
W2	*R.J.McKenzie 190/1*	+	
W3	*R.J.McKenzie 190/2*	+	
W4	*R.J.McKenzie 190/3*	+	
W5	*R.J.McKenzie 203*	+	
W6	*R.J.McKenzie 204*	+	
W7	*R.J.McKenzie 205*	+	+
W8	*R.J.McKenzie 374/1*	+	+
W9	*R.J.McKenzie 374/2*		+
W10	*R.J.McKenzie 374/3*		+
W11	*R.J.McKenzie 374/4*	+	
W12	coll. J. Whillans	+	
Putative *Leucogenes grandiceps* × *Raoulia mammillaris*:			
W13	*R.J.McKenzie 206/2*	+	

Table 5.1. Specimens of putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and between *L. grandiceps* and *R. mammillaris*, available for study in this thesis.

and *W10* in cultivation failed, so continuous vegetative characters were unavailable for these plants. The characters utilised are described in Chapter 3.

Herbarium specimens of putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* collected from other localities were examined to gain an insight into the frequency, geographical distribution and variation among natural putative hybrids between the two species. Capitula were absent from all of the specimens, so only vegetative characters were available for determinations.

5.2.3 Histology

Transverse hand sections were taken from the midpoint of the lamina of FAA-preserved leaves with the aid of a stereo microscope. The sections were rinsed in distilled water for 30 min and cleared in 8 N NaOH for 1 h to overnight. After rinsing in distilled water for 30 min, the sections were stained briefly in 0.05 % aqueous toluidine blue, mounted in water and viewed with an Olympus BW2 microscope.

5.2.4 Cytology

Immature capitula from two putative hybrids were collected from the study site on 20 December 1998 and fixed in 3 parts acetic acid: 1 part ethanol at approximately 3 p.m. The subsequent method was identical to that used in Chapter 4 (see p. 90).

5.2.5 Analyses of morphological data

A multiple-method approach was employed, but the approach differed slightly from that of Chapter 4. All available characters were recorded for *all* sympatric species, so only a single data set was compiled. For each method in which all sympatric species could be included in the data set (i.e., CDA, cluster analysis, HYWIN, MDS and split decomposition), the complete data set was analysed. The species least likely to be parents were then excluded and the reduced data set analysed. Support for which species to include in character counts and character indices was obtained from the other analytic methods. The characters included in each analysis are summarised in Appendix 5. For character codes, also see Table 5.5–Table 5.8 (following pp. 211–218).

Relationship between leaf dimensions and number of leaf traces in *W3* and *W8*

Student's *t* tests, Wilcoxon rank-sum tests and an ANOVA were performed using the S-PLUS 4.5 computer program (MathSoft, 1997) to test the relationship between leaf dimensions and the number of leaf traces in the putative hybrids *W3* and *W8*.

Character count

Eighteen continuous and 27 discrete characters discriminating the putative parental species were included in the data set. Standard-deviation intervals centred on the mean, and first and third quartiles centred on the median, were used to determine the parental limits for continuous characters. Five characters (45, 55, 62, 64 and 95, see Appendix 5) were included only in counts employing quartile limits, as the parental limits derived from standard-deviation intervals overlapped.

Character index

Seventeen continuous and 31 discrete characters that discriminated the putative parental species were selected. Where necessary, characters were recoded so that *L. grandiceps* always received the maximum value for each character and *R. eximia* the minimum value, but occasionally a putative hybrid received the most extreme value. Character indices were derived separately from continuous and discrete, vegetative and floral, and mixed characters.

Indices were recalculated following inclusion in the data set of an additional six continuous characters (50, 55, 65, 73, 88 and 90, see Appendix 5), in which the range of means for the putative parental species overlapped.

Canonical discriminant analysis

Up to 37 continuous characters were analysed. The distribution of each character and presence of outliers were assessed with normal probability plots and box plots. Characters with outliers or a non-normal distribution were log or square-root transformed (see Appendix 5). However, after transformation characters 10, 44, 47, 48, 49, 52, 56, 72, 76, 91 and 92 still contained outliers. Analysis of the complete data set allowed only *W7* and *W8* to be compared with the sympatric species, as missing data is not allowed. Exclusion of characters with missing data allowed the inclusion of the other putative hybrids in separate analyses: *W7*, *W8*, *W9* and *W10* (with characters 4, 5, 6, and 10 excluded); and *W1*, *W2*, *W3*, *W4*, *W5*, *W6*, *W7*, *W8*, *W11*, *W12* and *W13* (with all floral characters excluded). In each case, separate analyses of the complete data set and subsets created by exclusion of characters with outliers were performed. Only the first, second and third canonical variates were evaluated.

Calculation of dissimilarities

As in case study 1, a matrix of dissimilarities was generated with the S-PLUS 4.5 computer program (MathSoft, 1997) using Gower's (1971) general coefficient of similarity and the Phenetic Library developed by Dr Aaron Wilton, Landcare Research Ltd. Separate dissimilarity matrices were calculated from the complete data set and from a reduced data set with non-parental species excluded.

Cluster analysis

Only agglomerative hierarchical clustering with group-average linkage was employed in this case study.

Split decomposition

Analyses were performed as in case study 1. Analyses of the complete data set were extremely slow. To achieve more rapid analyses, the data for each species was combined into a single 'OTU' per species; for each species an overall mean was calculated for each continuous character. Dissimilarities were calculated as described above.

5.2.6 Experimental crosses

Plants of both putative parental species did not flower in cultivation, so experimental pollinations were performed using field-collected specimens. Flowering shoots with roots attached were replanted in pots containing perlite and placed in the insect-proof cage in the glasshouse. Pollen germination on the stigma was estimated in three crosses (two *L. grandiceps* × *R. eximia* crosses employing capitula from different *L. grandiceps* plants, and one *R. eximia* × *L. grandiceps* cross). The proportion of germinated grains on five to seven florets per cross, and the number of retracted styles and filled cypselas, were recorded.

5.3 Results

5.3.1 Pollen stainability

In *W9* 81.3 % of the pollen grains were normally developed, as indicated by staining with Alexander's differential stain. Normal pollen grains were filled with red-staining cytoplasm and were 20–25 μm in diameter. In some abnormal grains the cytoplasm stained strongly but was visibly shrunken from the pollen wall, while other grains were markedly smaller and contained little or no stainable cytoplasm. Only 1.2 % of the pollen grains from five plants of *L. grandiceps* were abnormal. In one plant of *R. eximia* 20.2 % of the pollen grains were abnormal, but in three other plants only 0.7 % were abnormal.

5.3.2 Meiotic pairing in microsporocytes

In *W9* florets were 0.8–1 mm long when the microsporocytes underwent the meiotic division. The stage of meiosis varied from interphase I to telophase II in these florets and often among anthers in the same floret. Microsporocytes at diakinesis were rarely observed. Determination of the number of chromosome pairs at this stage was difficult, as in most microsporocytes the chromosome pairs were rather fuzzy. In one microsporocyte 13 bivalents and two univalents were discernible (Plate 8 A p. 199). Interpretation of another microsporocyte was more equivocal with more than 14 chromosomal entities discernible (Plate 8 B). At metaphase I the chromosomes were usually closely associated and regularly aligned across the equator of the spindle (Plate 8 C), so unequivocal discrimination of all chromosome pairs was not possible. Occasionally two chromosomal bodies were positioned irregularly away from the equator (Plate 8 D & E). Observation of microsporocytes at subsequent stages also indicated a high regularity of meiosis. A chromosomal bridge at telophase I was occasionally observed (Plate 8 F). Micronuclei were rarely observed and usually tetrads were observed at telophase II.

Plate 8. Meiosis in microsporocytes of *W9*, a putative hybrid between *Leucogenes grandiceps* and *Raoulia eximia*.

A, Diakinesis, microsporocyte with probable 13 bivalents and two univalents. The arrow points to the closely associated univalents.

B, Diakinesis, microsporocyte with more than 14 chromosomal entities. The arrow indicates cellular debris.

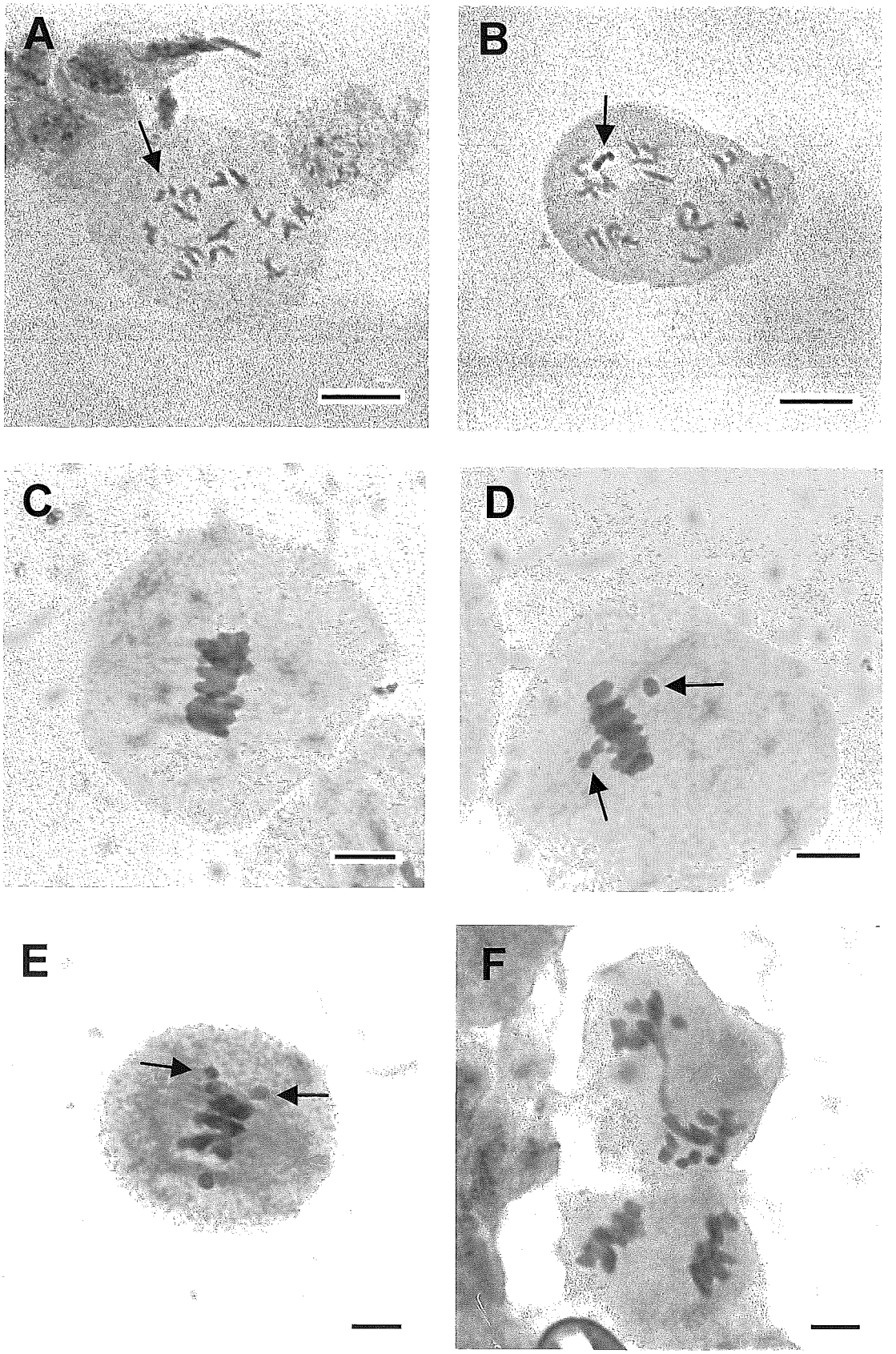
C, Metaphase I, showing regular distribution of chromosomes across the equator of the spindle.

D, Metaphase I, irregular positioning of two chromosomal entities away from the equator of the spindle (indicated by arrows).

E, Metaphase I, irregular positioning of two chromosomal entities away from the equator of the spindle (indicated by arrows).

F, Upper microsporocyte: late anaphase I, chromosome bridge. Lower microsporocyte: telophase I, showing regular distribution of chromosomes at the poles.

Scale = 10 μm.


A
B
C
D
E
F

5.3.3 Morphology of the putative hybrids and all sympatric gnaphalioid species

The morphological characters discriminating the sympatric species and putative hybrids are summarised in Tables 5.6–5.8 (following pp. 211–214).

Growth form

As described in the first case study (pp. 131–132), *A. bellidioides* had a mat-forming growth habit (Plate 9 A p. 215). The nonflowering shoots were prostrate with reduced leaves and arose from older wood near or below ground level. Flowering shoots initially assumed a prostrate orientation but the shoot tips became orthotropic following the intiation of capitula. The capitula-bearing shoots were morphologically distinct with narrow, acute, bracteate leaves and a solitary terminal capitulum.

Leucogenes grandiceps (Plate 9 B) was similar to *A. bellidioides* in growth form but the flowering shoots had an erect to partially decumbent orientation prior to the appearance of capitula (i.e., at most only the base of the shoot was prostrate) and so a distinct change in tropism did not occur prior to flowering. The nonflowering shoots were borne from older wood at or below ground level and were more readily distinguishable from flowering shoots than in *A. bellidioides*. Older plants can form distinct mats, but many plants at the site formed small clumps or more open patches.

Raoulia eximia and *R. mammillaris* formed cushions with tightly packed shoots (Plate 9 C & E). The cushions of *R. eximia* are extremely hard and impenetrable, even in cultivation, but *R. mammillaris* grows softer in cultivation than in the field. In both species the shoots at the perimeter of the plant were prostrate while shoots in the centre of the plant were erect, but morphologically distinct non-flowering shoots and flowering shoots were not produced.

The mat-forming *Raoulia* species (*R. grandiflora* and *R. subsericea*, Plate 9 D & F) produced morphologically distinct, prostrate non-flowering shoots and erect flowering shoots. The internodes were longer and the leaves somewhat smaller on the prostrate shoots. These species differed from *A. bellidioides* in that the flowering shoots were always autotropic (i.e., the tropism does not change prior to the appearance of capitula).

The putative hybrids had a cushion-forming growth habit and lacked distinct prostrate and erect shoots, but in all plants the shoots were not as tightly packed as in *R. eximia* and *R. mammillaris* (Plate 10 A–D p. 216).

Leaf morphology

The putative hybrids were variable in terms of leaf dimensions, leaf shape and leaf apex shape. The leaf morphology of *A. bellidioides*, *R. grandiflora* and *R. subsericea* was distinctive and suggested no affinity with the putative hybrids.

The leaf shape varied among the putative hybrids from oblong to weakly obovate (Figure 5.1 A–K p. 219). The leaves were obovate in *A. bellidioides* and *R. mammillaris*, oblong to weakly obovate in *L. grandiceps*, lanceolate in *R. grandiflora* and linear in *R. subsericea*. The leaf apex varied among the putative hybrids from obtuse to rounded. *Raoulia eximia* was the only sympatric species in which the leaf apex was rounded. The leaf apex was acute in *R. grandiflora* and obtuse in the other sympatric species. A mucro up to 10 μm long was present on the leaves of all putative hybrids except *W8* and *W11* and was plane with the lamina. A mucro was present in three sympatric species. In *A. bellidioides* the mucro was 50–65 μm long and was usually upturned, whereas in *L. grandiceps* and *R. subsericea* the mucro was shorter (10–15 μm and 8–12 μm respectively) and plane with the lamina.

Overall, the leaves of the putative hybrids were intermediate in dimensions between *L. grandiceps* and *R. eximia*, but exhibited notable variation between individuals and tended to be more similar to *L. grandiceps* in length (Figure 5.2 p. 220). The putative hybrids' leaves were similar in length to, but broader than, the leaves of *R. subsericea*. The leaves of *R. eximia* and *R. mammillaris* were smaller than those of the putative hybrids. The leaves of *A. bellidioides*, *L. grandiceps* and *R. grandiflora* were considerably larger than those of the other species.

The lamina and petiole were clearly visible in the shoot tips in *A. bellidioides*, *L. grandiceps* (Plate 11 A p. 217), *R. grandiflora* and *R. subsericea*. The clothing trichomes concealed the apex of *R. eximia* leaves (Plate 11 B), but the leaf apices were discernible in *R. mammillaris* and the putative hybrids (Plate 11 C & D).

The leaf sheath enclosed approximately 33–50 % of the stem in the putative hybrids, 75 % of the stem in *A. bellidioides*, 50 % of the stem in *L. grandiceps* and less than 50 % in the *Raoulia* species. The leaf sheath extensions extended over 75 % of the length of the internode in *A. bellidioides*, but the internodes were extremely compressed in the other species and the putative hybrids.

Leaf trichomes

The structure of the clothing trichomes provided strong evidence that *R. eximia* and *R. mammillaris* were possible parents of the putative hybrids. The form of glandular trichomes on the leaves suggested no affinity existed between the putative hybrids and *A. bellidioides* and *R. subsericea*.

The density of clothing trichomes on the adaxial lamina surface ranged from sparse to absent in *R. subsericea* to moderate in *A. bellidioides* and dense in the other species and the putative hybrids. On the abaxial leaf surface the density was moderate in *R. subsericea* and dense in all other species and putative hybrids. Type A clothing trichomes were present on the leaves of all species and putative hybrids except *R. eximia*. Type B clothing trichomes were present on both lamina surfaces in *R. eximia*, *R. mammillaris* and most putative hybrids. The terminal cells of type A trichomes were usually interwoven and appressed to the leaf surface, forming a felt-like indumentum, but on the petiole some terminal cells projected laterally beyond the leaf margin, notably in *R. mammillaris*. The width of the terminal cells of both types of clothing trichome varied considerably in the putative hybrids (e.g., 10–30 μm wide in *W2*), *Raoulia mammillaris* (6–47 μm wide) and *R. eximia* (15–37 μm). The terminal cells were narrower (not exceeding 18 μm wide) and less variable in width in the other sympatric species. *W13* was unusual among the putative hybrids in that the clothing trichomes had one or two basal cells, a character shared with *A. bellidioides* and *R. mammillaris*. Most putative hybrids and the other sympatric species had two or three basal cells (Figure 5.3 A–D p. 220), although the number of cells predominating was variable. The total length of the basal cells was generally 40–60 μm long in the putative hybrids and was similar to that of *L. grandiceps* and *R. eximia*. The basal cells were shorter in *R. mammillaris* and longer in *A. bellidioides*, *R. grandiflora* and *R. subsericea*. In most putative hybrids the terminal cells were predominantly appressed on the adaxial surface, but some were not appressed (particularly on the abaxial surface) and projected beyond the leaf apex. In *R. eximia* the terminal cells of type B clothing trichomes were not appressed to the lamina surface and projected prominently beyond the leaf apex, but in *R. mammillaris* the terminal cells of some trichomes were appressed and did not project notably beyond the lamina apex. The terminal cells were usually straight in *R. eximia* but were often somewhat undulate or distorted in *R. mammillaris* and the putative hybrids.

Type A glandular trichomes were present on the leaves of each sympatric species and the putative hybrids. These trichomes were consistently biseriate except in *R. grandiflora*, in

which they were almost exclusively uniseriate. The trichomes of *R. grandiflora* were also thinner walled and more translucent than those of the other species and the putative hybrids. The glandular trichomes of the putative hybrids were comparable in morphology and dimensions to those of *L. grandiceps*, *R. eximia* and *R. mammillaris*, although those of *W13* were at the lower end of the size range. These trichomes were extremely long (up to 234 μm in *R. eximia*) and of similar width throughout their length. The terminal cells were oblong, not swollen and up to 46 μm long. The type A glandular trichomes of *A. bellidioides* and *R. subsericea* were considerably shorter (up to 89 μm long). The terminal cells were oblong-oval and up to 18 μm long in *A. bellidioides*, but were more oblong and up to 35 μm long in *R. subsericea*.

Type B glandular trichomes were present on the leaves of *A. bellidioides* only. These trichomes were 100–175 μm long and 40–75 μm wide at the base, and were common on the adaxial lamina surface and leaf margins.

Leaf anatomy

The leaf anatomy of the putative hybrids was very uniform and most similar to *L. grandiceps*, *R. eximia* and *R. mammillaris*. *W13* was indistinguishable from the other putative hybrids with respect to lamina anatomy. The anatomy of *A. bellidioides* leaves was distinctive among the sympatric species and suggested little affinity between this species and the putative hybrids.

Anaphalioides bellidioides was unique in possessing a dorsiventral lamina with well-differentiated adaxial palisade and abaxial spongy chlorenchyma layers. In all putative hybrids and the other sympatric species the chlorenchyma was differentiated into three layers. The layers were well differentiated in *L. grandiceps* and *R. subsericea* but less differentiated in the other *Raoulia* species and most putative hybrids. In all plants palisade chlorenchyma was present on the adaxial side of the leaf. The mesophyll on the abaxial side of the leaf was well differentiated into palisade chlorenchyma in *R. subsericea* and *W2*, but only weakly palisade-like in the other putative hybrids, *R. eximia*, *R. grandiflora* and *R. mammillaris*. The abaxial mesophyll cells were oval and not differentiated into palisade chlorenchyma in *L. grandiceps*. The central chlorenchyma was most distinct in *L. grandiceps* (in which the cells were oblong and up to 80 μm wide) and *R. subsericea* (in which the cells were predominantly oval and up to 45 μm wide). The central chlorenchyma was less differentiated in the putative

hybrids. The cells varied in shape from oval to oblong and the maximum cell width ranged from 30 μm in *W12* to 60 μm in *W1* and *W8*. The central chlorenchyma was least differentiated in *R. eximia*, *R. grandiflora* and *R. mammillaris*. In these species the cells were oval to oblong-oval and up to 25 μm wide. Chloroplasts were common in the central chlorenchyma in *R. grandiflora* but in all other plants were more sparse than in the adaxial and abaxial chlorenchyma.

Two sclerenchyma fibres were observed on the adaxial side of the midvein in *W12*, but sclerenchyma was not observed in any other putative hybrid. A large sclerenchyma cap, exceeding the width of the vascular bundle, was present on the adaxial side of the midvein in *R. eximia* and of all veins in *R. grandiflora*. A smaller sclerenchyma cap, similar in width to the vascular bundle, was present on the abaxial side of the midvein in *R. mammillaris*. Idioblastic sclereids were present in the petiole on either side of the midrib in *R. eximia* and *R. mammillaris*, but were not observed in the putative hybrids.

In all putative hybrids, the adaxial and abaxial epidermal cells were of similar height, stomata were present on both surfaces and the guard cells were level with adjacent epidermal cells. The only sympatric species to differ in these characters were *A. bellidioides*, in which the adaxial epidermal cells were higher and stomata were present only on the abaxial surface, and *R. mammillaris* and *R. subsericea*, in which frequently the guard cells were raised above adjacent epidermal cells.

Leaf venation

The leaves were uninervate in *R. eximia* and *R. mammillaris* and trinervate in *A. bellidioides*, *L. grandiceps*, *R. grandiflora* and *R. subsericea*. Among the putative hybrids the number of leaf traces was variable, even on the same shoot. Leaves with one, two or three primary nerves were observed in most putative hybrids, but uninervate leaves were not observed in *W1*, *W8* and *W11*, trinervate leaves were not observed in *W13*, and only trinervate leaves were observed in *W9* (for which cultivated plants were not available for study). Occasionally, one of the lateral primary nerves was unconnected with the lamina venation and ended blind in the petiole (e.g., Figure 5.1 G & H p. 219). In *W3* and *W8* the number of leaf traces was not associated with differences in leaf dimensions (Table 5.2–5.4 pp. 205–206). The venation types were not regularly distributed on a shoot, although one type tended to predominate, and were not related to the presence or absence of a capitulum.

	n	range (mm)	mean (mm)	variance	Student's *t* test		Wilcoxon rank-sum test	
					t	*P*	*Z*	*P*
Leaf length								
two nerves	7	5.4–6.1	5.70	0.0576	-0.4151	0.6829	-0.5975	0.5502
three nerves	13	5.2–6.1	5.76	0.0927				
Leaf width								
two nerves	7	2.8–3.2	2.93	0.0282	0.1620	0.8731	-0.0400	0.9681
three nerves	13	2.3–3.4	2.91	0.0844				
Length:width ratio								
two nerves	7	1.8–2.1	1.95	0.0063	-0.6611	0.5169	-0.1192	0.4370
three nerves	13	1.8–2.4	1.99	0.0238				

Table 5.2. Tests for differences in the dimensions of leaves with differing number of leaf traces in *W8*, a putative hybrid between *Leucogenes grandiceps* and *Raoulia eximia.*

leaf type	n	range (mm)	mean (mm)	variance
Leaf length				
one nerve	7	4.7–5.6	5.13	0.1378
two nerves	10	4.7–5.8	5.16	0.2133
three nerves	8	4.7–6.1	5.36	0.1501
Lamina width				
one nerve	7	2–2.8	2.46	0.0723
two nerves	10	2.2–2.8	2.50	0.0350
three nerves	8	2.3–2.7	2.46	0.0431
Length:width ratio				
one nerve	7	1.7–2.7	2.12	0.1317
two nerves	10	1.7–2.4	2.08	0.0688
three nerves	8	1.7–2.4	2.19	0.0479

Table 5.3. Dimensions of leaves with differing number of leaf traces in *W3*, a putative hybrid between *Leucogenes grandiceps* and *Raoulia eximia.*

Source	df	Sum of squares	Mean square	*F*	*P*
leaf length	1	0.7498	0.7498	1.0931	0.3104
leaf width	1	0.0726	0.0726	0.1059	0.7488
leaf length:width ratio	1	1.0595	1.0595	1.5446	0.2308
leaf length*leaf width	1	0.2479	0.2479	0.3614	0.5556
leaf length*leaf l:w ratio	1	0.2739	0.2739	0.3994	0.5358
leaf width*leaf l:w ratio	1	0.0555	0.0555	0.0809	0.7795
leaf length*leaf width*leaf l:w ratio	1	0.8403	0.8403	1.2251	0.2838
Residuals	17	11.6605	0.6859		

Table 5.4. ANOVA of relationship between leaf dimensions and the number of leaf traces in *W3*, a putative hybrid between *Leucogenes grandiceps* and *Raoulia eximia.* The residual standard error was 0.8282.

The laminar venation pattern of the putative hybrids was very simple and exhibited relatively little variation between individuals (Figure 5.1 A–D p. 219). The higher-order veins had a pronounced looping pattern and up to quarternary nerves were present. Areolar veinlets and free-ending veins were generally uncommon. The degree of higher-order nerve branching was not influenced by the number of primary nerves. The venation pattern was very similar to that of *R. eximia*, *R. mammillaris* and *R. subsericea*. *Anaphalioides bellidioides* was distinct among the sympatric species in possessing greater higher-order nerve branching, resulting in a reticulate venation pattern, and areolar veinlets were more frequent than in the other species. The venation of *R. grandiflora* leaves was also distinctive in possessing few higher-order veins and in the essentially parallel orientation of the higher-order veins.

The midrib was raised on the abaxial surface in *A. bellidioides*, but was plane with the leaf surface in the other species and the putative hybrids. In all plants the lateral primary nerves and higher-order veins were not raised.

Floral morphology

Leucogenes grandiceps was the only sympatric species to produce multicapitulate inflorescences, which comprised 5–11 capitula (Plate 12 A p. 218). In *Raoulia eximia* (Plate 14 B) and the other sympatric species, a solitary capitulum was produced on each flowering shoot. In the four putative hybrids for which capitula were available for study, solitary or multicapitulate inflorescences were borne on each flowering shoot (Plate 12 D–F). The

capitula-bearing shoots were not distinctly elongated, although the capitula were borne slightly above the nonflowering shoots, and the leaves of nonflowering and flowering shoots were morphologically identical. Elongated, morphologically distinct flowering shoots were present in *A. bellidioides* and *L. grandiceps*. The leaves on the lower portion of the flowering shoot were partially appressed to the stem in both species. These leaves were intermediate in morphology between involucral bracts and leaves on nonflowering shoots in *A. bellidioides*, but in *L. grandiceps* were morphologically similar to leaves on nonflowering shoots.

In *W7* the leaves surrounding the capitulum were morphologically identical to leaves from nonflowering shoots except that the clothing-trichome terminal cells were longer and protruded further beyond the leaf apex. In *W8* and *W10* the subtending leaves were slightly longer and narrower than true leaves below. In *W9* the subtending leaves were similar in length to the involucral bracts. In *L. grandiceps* each capitulum was subtended by a large trinervate bract, which was considerably longer than the lower leaves on flowering shoots and leaves on non-flowering shoots. Collectively, the bracts formed a showy 'pseudoray' surrounding the cluster of capitula (Plate 12 A). In addition, the indumentum on the bracts was more dense and less appressed than on leaves. In the other species the leaves subtending the capitulum were morphologically intermediate between involucral bracts and leaves on nonflowering shoots, and were either similar in length to involucral bracts (*A. bellidioides*) or true leaves (*R. eximia*, *R. mammillaris* and *R. subsericea*).

At anthesis the capitula of *A. bellidioides* were pedunculate, but in the putative hybrids and the other sympatric species the capitula were sessile. The dimensions of the terminal capitulum in the putative hybrids were similar to the capitulum dimensions of *R. eximia* and *R. mammillaris* (Table 5.8 p. 214). The capitula were longer in all other sympatric species, similar in width in *R. subsericea*, slightly broader in *L. grandiceps* and *R. grandiflora*, and much broader in *A. bellidioides*.

The numbers of female and hermaphrodite florets per capitulum in the putative hybrids were similar to *R. grandiflora* and were intermediate between *L. grandiceps* and *R. eximia* (Figure 5.4 p. 221). *Raoulia mammillaris* was extremely similar to *R. eximia* in these characters. *Raoulia subsericea* had a similar number of hermaphrodite florets per capitulum but female florets were much more numerous. The numbers of female and hermaphrodite florets in *A. bellidioides* capitula greatly exceeded those in all other plants.

Receptacle dimensions were variable among the putative hybrids (Figure 5.5 p. 221). The mean receptacle diameter ranged from 0.8 mm in *W7* and *W8* to 1.2 mm in *W10*, and receptacle height ranged from 0.19 mm in *W8* to 0.55 mm in *W10*. The receptacle diameter ranged from 0.5–0.75 mm in *R. eximia* and *R. mammillaris* to 2–3.2 mm in *A. bellidioides*. The shape of the receptacle was conical in *A. bellidioides*, convex in the putative hybrids, *L. grandiceps* and *R. eximia*, and flat or convex in *R. grandiflora*, *R. mammillaris* and *R. subsericea*. The receptacle surface was scrobiculate in *Anaphalioides bellidioides* and *R. mammillaris*, foveolate in the putative hybrids, *R. eximia* and *R. grandiflora*, and fimbrillate in *R. subsericea*. The receptacle varied among plants in *L. grandiceps* from fimbrillate to foveolate or weakly scrobiculate. Receptacle scales were absent in all plants.

The inner involucral bracts were considerably longer in *A. bellidioides* and *R. grandiflora* than in the putative hybrids and other sympatric species (Table 5.8 p. 214). The involucral bracts were broadest in *A. bellidioides* (up to 2.3 mm wide) and narrowest in *R. eximia* (0.4–0.9 mm wide). The involucral bracts of the putative hybrids were intermediate in width between those of *L. grandiceps* and *R. eximia* (Figure 5.6 p. 222). The lamina was hygroscopic in *A. bellidioides*, *R. grandiflora* and *R. subsericea*, but not in the putative hybrids. The lamina colour ranged from white in *A. bellidioides*, *R. grandiflora*, *R. mammillaris* and *R. subsericea* to pale brown in *R. eximia* and the putative hybrids and blackish-brown in *L. grandiceps*. The lamina apex was acute in *W7*, *W9* and *W10* and acute to obtuse in *W8*. Among the sympatric species *R. eximia* was the only species to have consistently acute lamina apices (Figure 5.7 p. 222). Broad hyaline margins (200–325 μm wide) on the stereome were present in *L. grandiceps*, but in all putative hybrids and the other sympatric species the hyaline margins were narrow.

The upper corolla tube and base of the corolla lobes were crimson in the putative hybrids and *R. eximia* (Plate 12 B, D & F p. 218). The lower corolla tube was also crimson in some plants of *R. eximia*. The corolla lobe apex was translucent in *R. eximia* and *W8*, and yellow in *W7* (Plate 12F), *W9* and *W10*. In the other sympatric species the corolla lobes were white (*Raoulia mammillaris*, *R. subsericea*), greenish-yellow (*L. grandiceps*, Plate 12C) or pale green (*A. bellidioides*). The corolla tube of hermaphrodite florets was slightly longer than that of female florets in *W7* and *W8*, but were similar in length in all other plants (Table 5.8 p. 214). The corolla tube of female florets was of similar length in *R. eximia*, *R. mammillaris* and the putative hybrids and longer in the other species, particularly *R. grandiflora* and *R. subsericea*. The length of the corolla tube of hermaphrodite florets was similar in the putative hybrids to

that of *A. bellidioides*, *L. grandiceps*, *R. eximia* and *R. mammillaris*, but was notably longer in *R. grandiflora* and *R. subsericea*. The distance to the point of expansion of the corolla tube from its base in hermaphrodite florets was greater in *R. subsericea* but similar in all other plants. The width of the corolla tube in each floret type was similar in all species and putative hybrids.

The style arms of hermaphrodite florets were similar in length (predominantly 0.5–0.8 mm) in the putative hybrids, *A. bellidioides*, *L. grandiceps*, *R. eximia* and *R. grandiflora* (Table 5.8 p. 214). In *R. mammillaris* the style arms of most hermaphrodite florets were predominantly 0.2–0.5 mm long, but were occasionally shorter and in a single floret style arms were lacking. The style arms of hermaphrodite florets were notably longer (usually 0.8–1.1 mm long) in *R. subsericea*.

The pappus hairs of the female and hermaphrodite florets were morphologically identical in *A. bellidioides* and *R. grandiflora* but dimorphic in the putative hybrids and the other sympatric species (Figure 5.8 p. 223). In *R. subsericea* the female-floret pappus hairs were also dimorphic. The apical cells were obtuse in female florets and clavate in hermaphrodite florets of the putative hybrids, characters shared with *L. grandiceps* and most *R. mammillaris* plants. The apical cells were obtuse in hermaphrodite florets of two *R. mammillaris* plants but clavate in all other plants. The pappus hairs were distinctly flattened and broader below the apex in *R. eximia* but less distinctly flattened in the putative hybrids, *L. grandiceps* and *R. mammillaris*. The apical cell walls were uniformly thickened in the putative hybrids, *A. bellidioides*, *L. grandiceps* and *R. eximia*, and had reticulate thickening in the other species. Basal spines were sparse in *R. eximia*, *R. mammillaris* and *R. subsericea*. The angle of the basal spines varied from ascending in *R. mammillaris* to ascending to recurved in *R. eximia*. In the putative hybrids and the other species the spines were ascending or spreading only. The basal spines were up to 30 μm long in the putative hybrids, intermediate in length between *R. eximia* and the other sympatric species. The apex of at least some basal spines was acute in *L. grandiceps*, *R. eximia*, *R. mammillaris* and the putative hybrids, but always obtuse in the other species. The pappus hairs of both florets were similar in length in the putative hybrids, *L. grandiceps*, *R. eximia* and *R. mammillaris*, and markedly longer in *R. grandiflora* and *R. subsericea*.

The ovary of both floret types was smallest in *A. bellidioides* but similar in dimensions in the other sympatric species and the putative hybrids (Table 5.8 p. 214). In *R. mammillaris* the

ovary of some hermaphrodite florets was unusually short (as small as 0.53 mm long). In the putative hybrids ovary length of hermaphrodite florets did not vary notably. For both floret types the ovary length:width ratio was similar in all species and the putative hybrids. The ovary epidermal cells were rounded in *R. subsericea* and smooth in the other species and the putative hybrids.

Long twin hairs densely covered the ovary of both floret types in *L. grandiceps*, *R. eximia*, *R. grandiflora*, *R. mammillaris* and the putative hybrids. The terminal cells were obtuse and coherent to the apex in *R. grandiflora*, but acute and free at the apex (to varying degrees) in the other species and the putative hybrids. The twin hairs were longest in *R. eximia* and *R. mammillaris* and considerably shorter in *R. grandiflora* (Table 5.8 p. 214). The twin hairs of *L. grandiceps* and the putative hybrids were intermediate in length. The trichomes were 30–40 μm long and 18–23 μm wide and comprised two small basal cells and two oblong terminal cells (20–35 μm long × 7–12 μm wide). The ovary of both floret types was glabrous in *A. bellidioides*.

Table 5.5. Discrete vegetative characters for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* (*W1–W12*), and between *L. grandiceps* and *R. mammillaris* (*W13*), and all sympatric gnaphalioid species.

Key to characters: 1, growth form; 2, nonflowering shoot orientation; 3, distinct internodes on non-flowering and flowering shoots; 7, leaf shape; 8, leaf apex shape; 9, stem enclosure by petiole extensions; 11, mucro orientation; 12, leaf indumentum density (adaxial surface); 13, type A clothing trichomes on leaf; 14, type B clothing trichomes on leaf; 15, clothing trichome terminal cell appressed on adaxial lamina surface; 16, clothing trichome terminal cells on abaxial lamina surface; 17, number of clothing trichome basal cells; 21, leaf glandular trichomes, number of cell series; 24, type A glandular trichomes, terminal cell shape; 26, type B glandular trichomes on leaf; 27, midrib raised on abaxial leaf surface; 28, epidermis thickness; 29, stomata distribution on leaf; 30, lamina structure; 31, mesophyll differentiation; 32, spongy chlorenchyma; 33, maximum width of central chlorenchyma cells; 34, sclerenchyma on adaxial side of midrib; 35, sclerenchyma on abaxial side of midrib and major lateral veins; 36, idioblastic sclereids in petiole; 37, number of leaf traces. NA = not applicable; ND = data missing.

Character	*A. bellidioides* (six plants)	*L. grandiceps* (six plants)	*R. eximia* (ten plants)	*R. grandiflora* (eight plants)	*R. mammillaris* (nine plants)	*R. subsericea* (seven plants)	*W1*	*W2*	*W3*
1	mat	mat to subshrub	cushion	mat	cushion	mat	cushion	cushion	cushion
2	prostrate	decumbent to erect	prostrate to erect	prostrate	prostrate to erect	prostrate	prostrate to erect	prostrate to erect	prostrate to erect
3	present	absent	absent	absent	absent	absent	absent	absent	absent
7	obovate	oblong to obovate	oblong to obovate	lanceolate	obovate	linear	oblong to obovate	oblong to obovate	oblong to obovate
8	obtuse	obtuse	rounded	acute	obtuse	obtuse	obtuse	rounded	obtuse
9	> 50 %	≤ 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %
11	upturned	plane	NA	NA	NA	plane	plane	plane	plane
12	sparse to moderate	dense	dense	dense	dense	glabrous to sparse	dense	dense	dense
13	present	present	absent	present	present	present	present	present	present
14	absent	absent	present	absent	present	absent	absent	present	present
15	all	all	none	all	some	all	all	some	some
16	all	all	none	all	all	all	all	some	some
17	1–2	2–3	2–3	2–3	1–2	2–3	2–3	2–3	2–3
21	2	2	2	1	2	2	2	2	2
24	oblong-oval	oblong	oblong	oblong	oblong	oblong-oval	oblong	oblong	oblong
26	present	absent	absent	absent	absent	absent	absent	absent	absent
27	present	absent	absent	absent	absent	absent	absent	absent	absent
28	adaxial thicker	equal thickness	equal thickness	equal thickness	equal thickness	adaxial thicker	equal thickness	equal thickness	equal thickness
29	adaxial & abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial
30	dorsiventral	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial
31	well	well	moderate or poor	moderate or poor	moderate or poor	well	moderate or poor	well	moderate or poor
32	present	absent	absent	absent	absent	absent	absent	absent	absent
33	NA	up to 80 μm	up to 25 μm	up to 30 μm	up to 30 μm	up to 45 μm	up to 60 μm	up to 50 μm	up to 50 μm
34	absent	absent	broader	broader	absent	absent	absent	absent	absent
35	absent	absent	absent	absent	present	absent	absent	absent	absent
36	absent	absent	present	present	absent	absent	absent	absent	absent
37	three	three	one	three	one	three	two–three	one–three	one–three

Table 5.5 (continued).

Character	*W4*	*W5*	*W6*	*W7*	*W8*	*W9*	*W10*	*W11*	*W12*	*W13*
1	cushion	cushion	cushion	cushion	cushion	cushion	cushion	cushion	cushion	cushion
2	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect	prostrate to erect
3	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
7	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate	oblong to obovate
8	obtuse	obtuse	rounded	obtuse	rounded	rounded	obtuse	rounded	obtuse	obtuse
9	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %	< 50 %
11	plane	plane	plane	plane	NA	plane	plane	NA	plane	plane
12	dense	dense	dense	dense	dense	dense	dense	dense	dense	dense
13	present	present	present	present	present	present	present	present	present	present
14	present	absent	present	present	present	present	present	present	present	present
15	some	all	some	some	all	some	some	some	all	some
16	some	all	some	some	some	some	some	some	some	some
17	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	2–3	1–2
21	2	2	2	2	2	2	2	2	2	2
24	oblong	oblong	oblong	oblong	oblong	oblong	oblong	oblong	oblong	oblong
26	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
27	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
28	equal thickness	equal thickness	equal thickness	equal thickness	equal thickness	equal thickness	equal thickness	equal thickness	equal thickness	equal thickness
29	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial	abaxial
30	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial	equifacial
31	moderate or poor	moderate or poor	moderate or poor	moderate or poor	moderate or poor	moderate or poor	moderate or poor	moderate or poor	moderate or poor	moderate or poor
32	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
33	up to 50 µm	up to 45 µm	up to 50 µm	up to 40 µm	up to 60 µm	up to 35 µm	up to 40 µm	up to 40 µm	up to 30 µm	up to 50 µm
34	absent	absent	absent	absent	absent	absent	absent	absent	narrower	absent
35	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
36	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
37	one–three	one–three	one–three	one–three	two–three	three	one–three	two–three	one–three	one–two

Table 5.6. Discrete floral characters for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* and all sympatric gnaphalioid species.

Key to characters: 38, morphologically distinct flowering shoots; 39, transition from leaves to involucral bracts; 40, length of leaves subtending capitulum (A, similar to leaves on nonflowering shoots; B, intermediate between leaves and involucral bracts; C, similar to involucral bracts; D, longer than involucral bracts); 41, morphology of leaves subtending capitulum; 42, indumentum on leaves subtending capitulum projects beyond apex; 43, capitulum pedunculate; 53, receptacle type; 54, receptacle shape; 57, inner involucral bract, lamina colour; 58, lamina of inner involucral bracts hygroscopic; 59, inner involucral bract, shape of lamina tip; 60, inner involucral bract, gap colour; 61, inner involucral bract, hyaline margins on stereome; 66, lower corolla tube crimson at anthesis; 67, corolla lobe and upper corolla tube colour at anthesis; 68, corolla lobe apex coloration; 69, corolla lobe curvature; 70, crimson coloration in anthers; 71, style colour; 77, pappus hairs dimorphic between female and hermaphrodite florets; 78, female floret pappus hairs, apical cells distinctly protruding; 79, hermaphrodite floret pappus hairs, apical cells distinctly protruding; 80, female floret pappus hairs, shape of apical cells; 81, hermaphrodite floret pappus hairs, shape of apical cells; 82, pappus hair distinctly broader below apex; 83, type of wall thickening in pappus hair apical cells; 84, length of basal spines on pappus hairs; 85, angle of basal spines on pappus hairs; 86, pappus hair basal spine, apex shape; 93, ovary epidermis surface; 94, twin hairs on ovary of female and hermaphrodite florets; 96, ovary twin hairs, shape of terminal cells; 97, ovary twin hairs, fusion of terminal cells; 98, glandular trichomes on ovary of female and hermaphrodite florets. NA = not applicable; ND = data missing.

Table 5.7. Continuous vegetative characters recorded from cultivated plants of putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* (*W1–W12*), and between *L. grandiceps* and *R. mammillaris* (*W13*), and all sympatric gnaphalioid species. The range, mean and standard deviation are presented for each character.

Key to characters: 4, leaf length (mm); 5, maximum lamina width (mm); 6, leaf length:lamina width ratio; 10, mucro length (mm); 18, clothing trichome basal cell length (μm); 19, clothing trichome basal cell width (μm); 20, clothing trichome terminal cell width (μm); 22, leaf type A glandular trichome length (μm); 23, leaf type A glandular trichome width (μm); 25, leaf type A glandular trichome terminal cell length (μm). ND, no data available.

Table 5.8. Continuous floral characters for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* and all sympatric gnaphalioid species. The range, mean and standard deviation are presented for each character (continued overleaf).

Key to characters: 44, number of capitula per inflorescence; 45, capitulum length (mm); 46, capitulum width at midpoint (mm); 47, number of female florets per capitulum; 48, number of hermaphrodite florets per capitulum; 49, total number of florets per capitulum; 50, female: hermaphrodite floret ratio; 51, receptacle diameter (mm); 52, receptacle height (mm); 55, inner involucral bract length (mm); 56, inner involucral bract width (mm); 62, corolla tube length in female florets (mm); 63, corolla tube length in hermaphrodite florets (mm); 64, hermaphrodite-floret corolla tube, width below corolla lobes (mm); 65, point of expansion of corolla tube from base:total corolla tube length in hermaphrodite florets; 72, style arm length in hermaphrodite florets (mm); 73, pappus hair length in female florets (mm); 74, pappus hair length in hermaphrodite florets (mm); 75, number of apical cells in pappus hairs of female florets; 76, number of apical cells in pappus hairs of hermaphrodite florets; 87, female-floret ovary length (mm); 88, female-floret ovary width (mm); 89, female-floret ovary length:width ratio; 90, hermaphrodite-floret ovary length (mm); 91, hermaphrodite-floret ovary width (mm); 92, hermaphrodite-floret ovary length:width ratio; 95, ovary twin hair length (mm). NA = character not applicable.

Table 5.8 (continued).

Species / putative hybrid	Character													
	64	65	72	73	74	75	76	87	88	89	90	91	92	95
A. bellidioides (six plants)	0.51–0.75 0.64 ± 0.06	0.47–0.68 0.59 ± 0.04	0.48–0.78 0.6 ± 0.08	3.1-4.4 3.8 ± 0.3	3.4-4.8 4 ± 0.3	1–3 2.1 ± 0.6	1–3 2.2 ± 0.6	0.5-1 0.69 ± 0.13	0.2-0.33 0.27 ± 0.03	2–3.7 2.6 ± 0.4	0.55-0.88 0.7 ± 0.1	0.24-0.38 0.31 ± 0.03	1.5–3.1 2.3 ± 0.4	NA
L. grandiceps (six plants)	0.54–0.83 0.71 ± 0.09	0.55–0.68 0.61 ± 0.04	0.38–1 0.7 ± 0.18	2.7–3.8 3.1 ± 0.3	2.8–3.8 3.2 ± 0.2	1–5 2.8 ± 0.9	2–7 4 ± 1.2	0.7–1.2 0.96 ± 0.15	0.26–0.38 0.32 ± 0.02	2.3–3.7 3 ± 0.4	0.66–1.08 0.91 ± 0.11	0.25–0.4 0.32 ± 0.04	2.1–3.4 2.8 ± 0.3	0.18–0.78 0.52 ± 0.13
R. eximia (ten plants)	0.43–0.65 0.52 ± 0.05	0.52–0.78 0.66 ± 0.06	0.38–0.71 0.59 ± 0.07	2.3–3.3 2.9 ± 0.3	2.6–3.6 3.1 ± 0.2	2–6 3 ± 0.9	3–7 4.8 ± 1.2	0.6–1.25 1.01 ± 0.13	0.28–0.46 0.37 ± 0.04	1.9–3.8 2.8 ± 0.5	0.75–1.28 1.05 ± 0.14	0.28–0.53 0.38 ± 0.04	2–4.2 2.8 ± 0.4	0.4–1.17 0.9 ± 0.21
R. grandiflora (eight plants)	0.48–0.93 0.69 ± 0.11	0.45–0.73 0.6 ± 0.05	0.38–0.75 0.56 ± 0.08	4.1–5.6 4.8 ± 0.4	3.7–5.6 4.7 ± 0.5	1–6 3 ± 0.8	1–6 3.4 ±1	0.6–1.2 0.87 ± 0.11	0.25–0.48 0.36 ± 0.06	1.8–3.1 2.5 ± 0.3	0.7–1.3 0.92 ± 0.14	0.23–0.43 0.35 ± 0.05	2–4.5 2.7 ± 0.4	0.12–0.22 0.17 ± 0.02
R. mammillaris (nine plants)	0.45–0.73 0.57 ± 0.08	0.54–0.75 0.62 ± 0.04	0–0.6 0.37 ± 0.11	2.2-3.3 2.8 ± 0.3	2.4-3.6 3.1 ± 0.3	1–7 4 ± 1.3	3–10 6.7 ± 1.7	0.7-1.1 0.92 ± 0.11	0.25-0.48 0.36 ± 0.05	2–3.3 2.6 ± 0.4	0.53-0.95 0.77 ± 0.1	0.3-0.45 0.36 ± 0.04	0.7–2.9 2.2 ± 0.4	0.39–1.21 0.98 ± 0.18
R. subsericea (seven plants)	0.5-0.85 0.68 ± 0.08	0.65–0.85 0.75 ± 0.04	0.7-1.4 1 ± 0.11	4.2-5.7 4.9 ± 0.4	4.6-6.1 5.3 ± 0.4	1–4 2.3 ± 0.8	2–6 3.7 ± 1	0.6-1 0.8 ± 0.1	0.24-0.41 0.33 ± 0.04	1.8-3.5 2.5 ± 0.4	0.7-1.1 0.8 ± 0.1	0.25-0.48 0.37 ± 0.04	1.8-3.3 2.2 ± 0.3	NA
W7	0.66–0.78 0.73 ± 0.05	0.6–0.72 0.68 ± 0.04	0.71–0.89 0.83 ± 0.06	2.8–3 2.9 ± 0.1	3–3.2 3.1 ± 0.1	1–3 1.9 ± 0.7	2–4 3 ± 0.8	0.82–0.92 0.87 ± 0.03	0.28–0.35 0.32 ± 0.02	2.5–3 2.7 ± 0.02	0.78–0.87 0.82 ± 0.03	0.35–0.4 0.37 ± 0.02	2–2.4 2.2 ± 0.1	0.35–0.9 0.63 ± 0.2
W8	0.58–0.7 0.64 ± 0.05	0.6–0.72 0.66 ± 0.04	0.56–0.71 0.64 ± 0.06	2.5–2.8 2.7 ± 0.1	2.7–2.8 2.7 ± 0.1	2–4 2.7 ± 0.7	3–7 5 ± 1.3	1.05–1.15 1.11 ± 0.04	0.33–0.38 0.36 ± 0.02	2.8–3.4 3.1 ± 0.3	1.08–1.13 1.11 ± 0.02	0.38–0.43 0.4 ± 0.02	2.6–3 2.8 ± 0.1	0.28–0.97 0.65 ± 0.24
W9	0.53–0.65 0.6 ± 0.06	0.55–0.71 0.63 ± 0.05	0.75–0.85 0.78 ± 0.03	2.9–3.2 3 ± 0.1	2.8–3 2.9 ± 0.1	2–3 2.6 ± 0.5	2–4 2.9 ± 0.7	0.88–1 0.98 ± 0.04	0.35–0.38 0.36 ± 0.02	2.5–2.9 2.7 ± 0.1	0.88–1.08 0.98 ± 0.07	0.35–0.45 0.39 ± 0.04	2–2.8 2.5 ± 0.3	0.35–0.93 0.67 ± 0.19
W10	0.5–0.58 0.55 ± 0.03	0.57–0.64 0.59 ± 0.03	0.8–0.88 0.82 ± 0.03	3.1–3.5 3.3 ± 0.1	3.1–3.5 3.4 ± 0.2	2–5 3 ± 0.9	3–4 3.4 ± 0.5	1–1.18 1.09 ± 0.05	0.33–0.36 0.35 ± 0.01	3–3.5 3.1 ± 0.1	0.98–1.05 1 ± 0.03	0.31–0.38 0.34 ± 0.03	2.6–3.3 3 ± 0.3	0.3–0.89 0.68 ± 0.23

Plate 9. Gnaphalieae that occur at the Mt Hutt study site.

A, *Anaphalioides bellidioides*, Mount Cheeseman skifield (photo John Lovis)

B, *Leucogenes grandiceps.*

C, *Raoulia mammillaris.*

D, *Raoulia grandiflora.*

E, *Raoulia eximia.* (photo John Lovis).

F, *Raoulia subsericea.* (photo John Lovis).

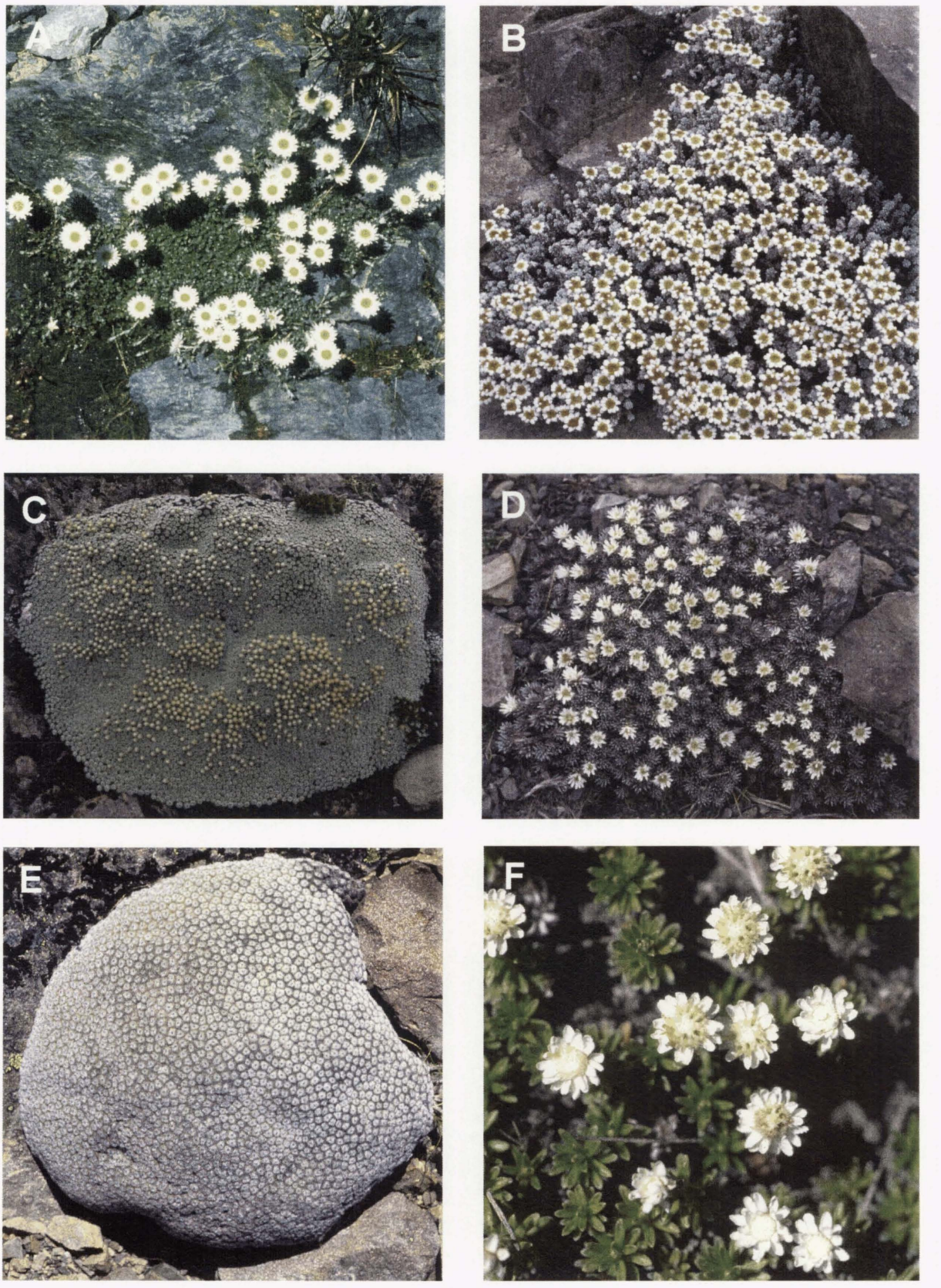

A
B
C
D
E
F

Plate 10. Growth form of putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*.

A, *W8* growing at the study site. Scale = 5 cm.

D, *W6* in cultivation. (photo Dougal Holmes). Scale = 1 cm.

E, *W1* in cultivation. (photo Dougal Holmes). Scale = 1 cm.

F, *W4* in cultivation. (photo Dougal Holmes). Scale = 5 mm.

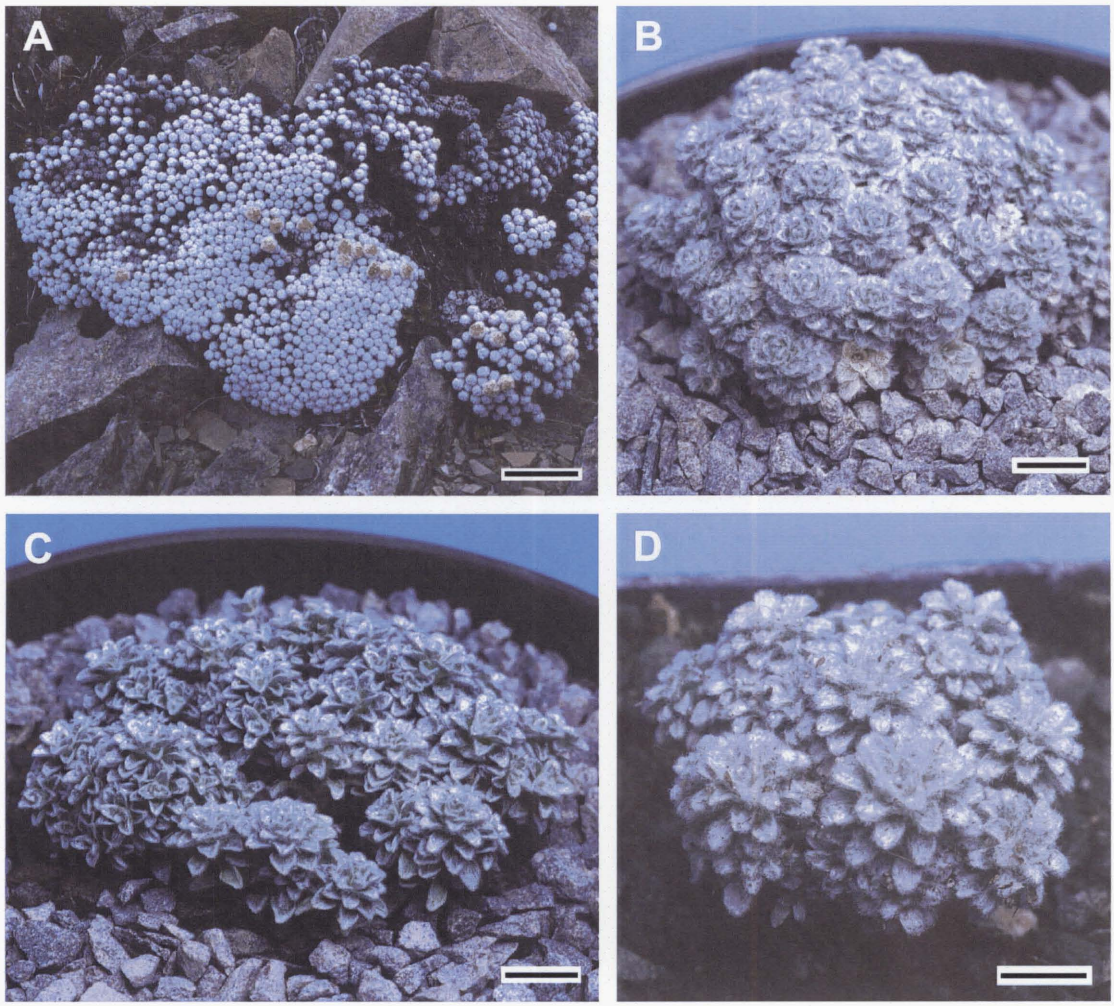

A
B
C
D

Plate 11. Vegetative shoot tip of *Leucogenes grandiceps*, *Raoulia eximia*, *R. mammillaris* and a putative hybrid between *L. grandiceps* and *R. eximia*.

A, *L. grandiceps*.

B, *R. eximia*.

C, *W7*.

D, *R. mammillaris*.

Scale = 2 mm.

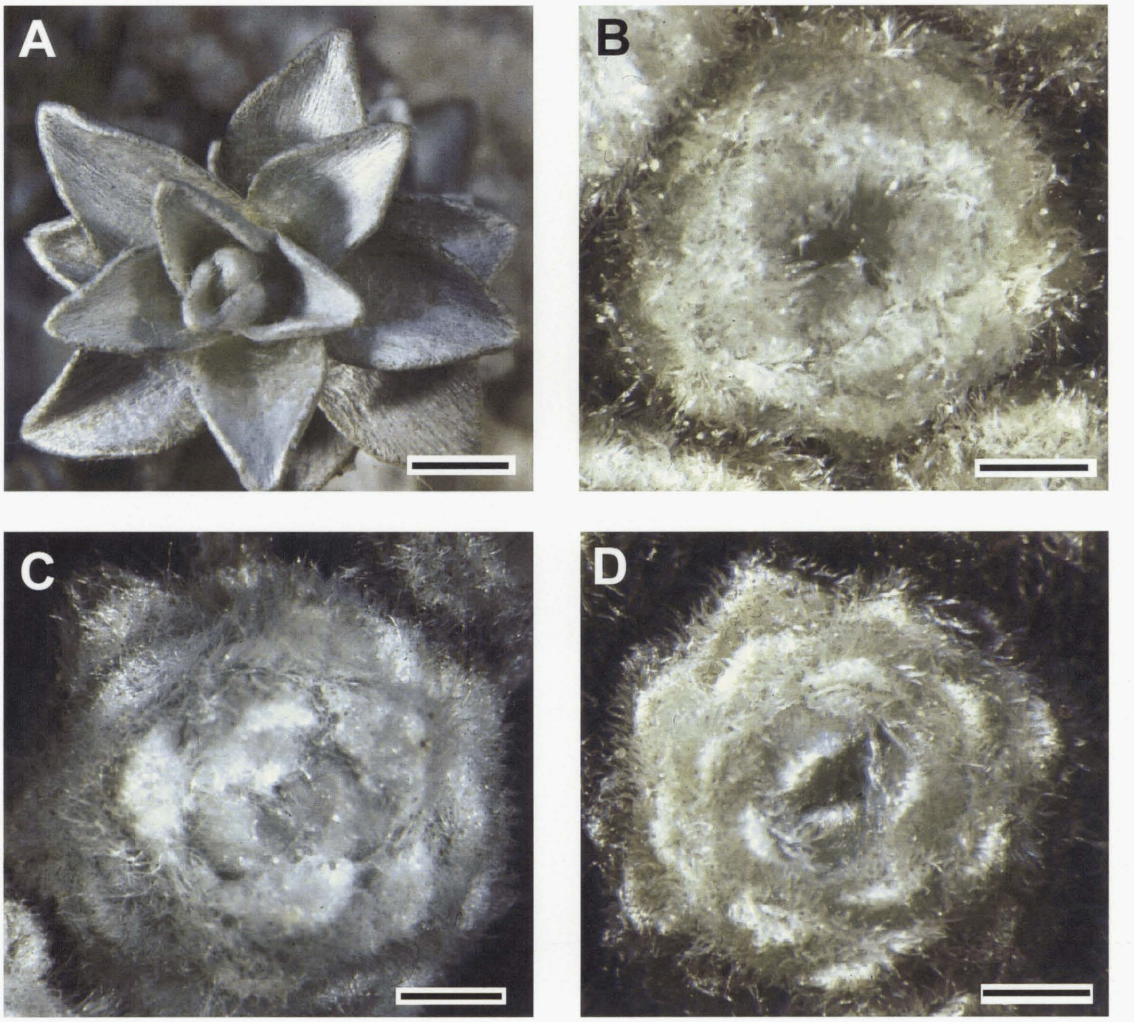

A
B
C
D

Plate 12. Capitula of *Leucogenes grandiceps*, *Raoulia eximia* and putative hybrids between the two species.

A, *L. grandiceps* inflorescence. Scale = 2.5 mm.

B, *R. eximia* capitulum. Scale = 1 mm.

C, *L. grandiceps* capitulum. Scale = 0.5 mm.

D, *W9* multicapitulate inflorescence. Scale = 2 mm.

E, *W10* multicapitulate inflorescence. Scale = 1.5 mm.

D, *W7* capitulum. Scale = 1 mm.

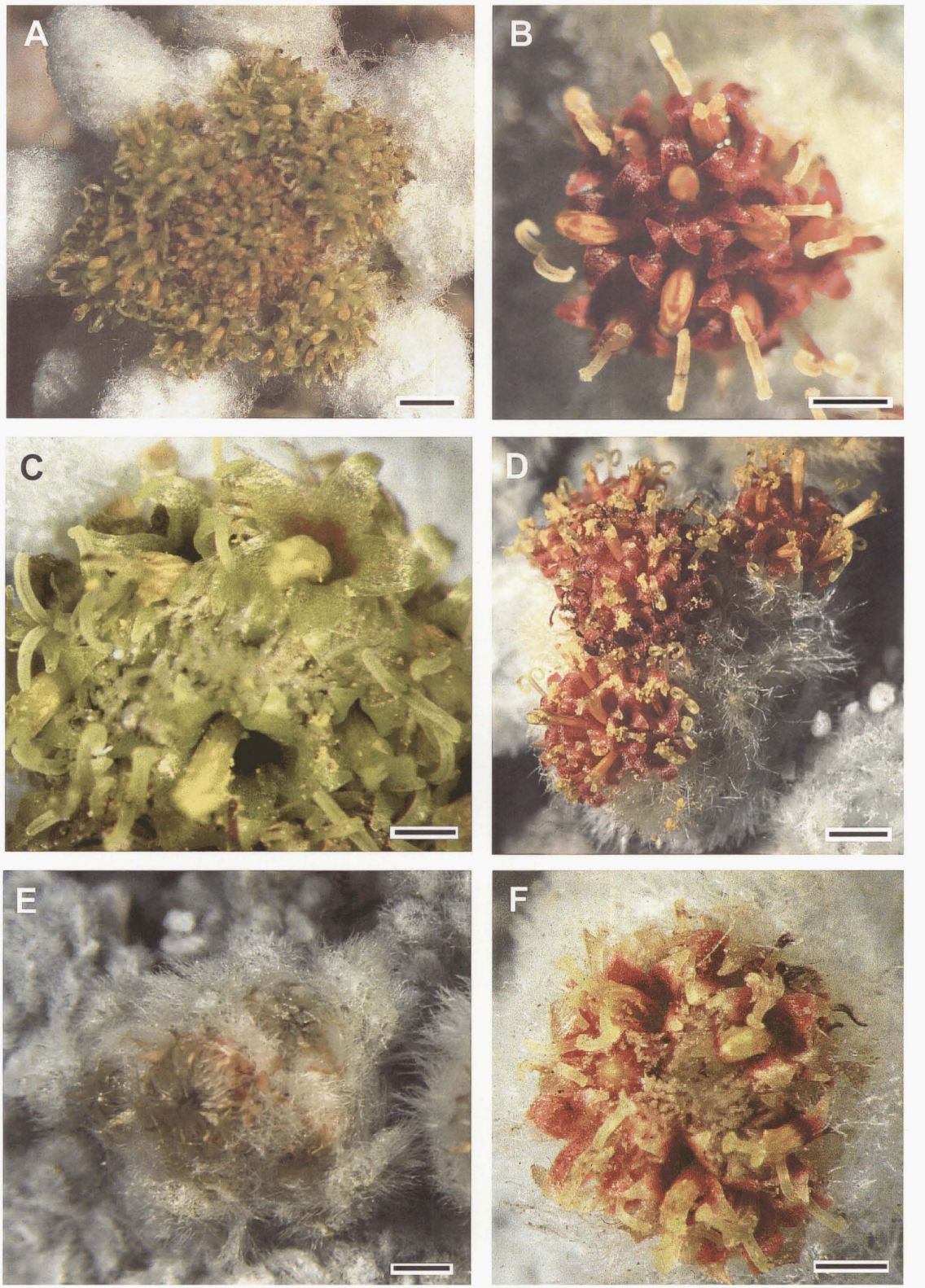

A
B
C
D
E
F

Figure 5.1. Leaf shape and venation of *Leucogenes grandiceps*, *Raoulia eximia*, *R. mammillaris* and putative hybrids between *L. grandiceps* and *R. eximia* (*W1–W12*) and between *L. grandiceps* and *R. mammillaris* (*W13*). All leaves illustrated are from cultivated plants.

A, *L. grandiceps*.

B, *R. eximia*.

C, *R. mammillaris*.

D, *W1*.

E, *W2*.

F, *W3*.

G, *W4*.

H, *W6*.

I, *W5*.

J, *W7*.

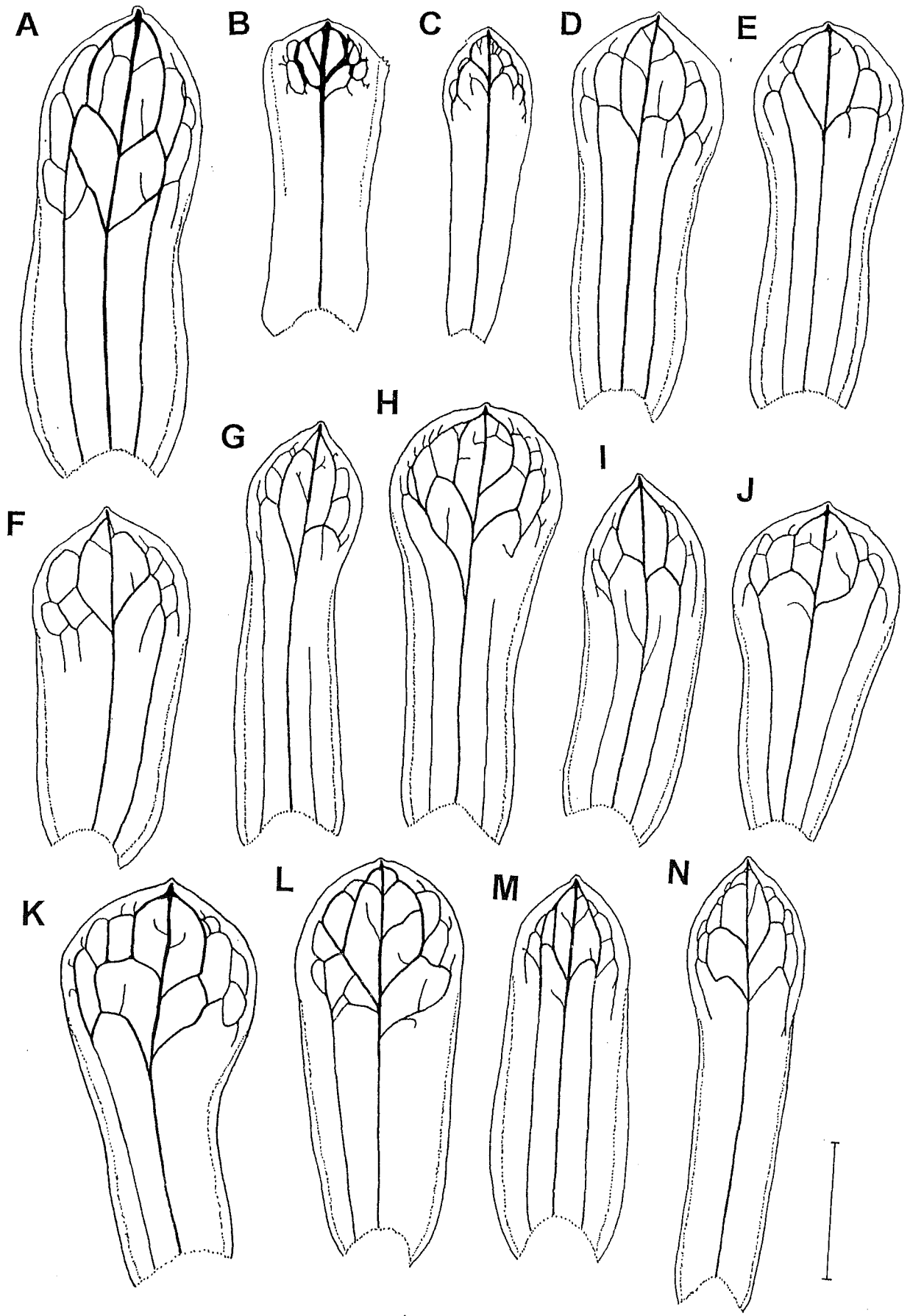
K, *W8*.

L, *W11*.

M, *W12*.

N, *W13*.

Scale = 2 mm.


A
B
C
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E
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G
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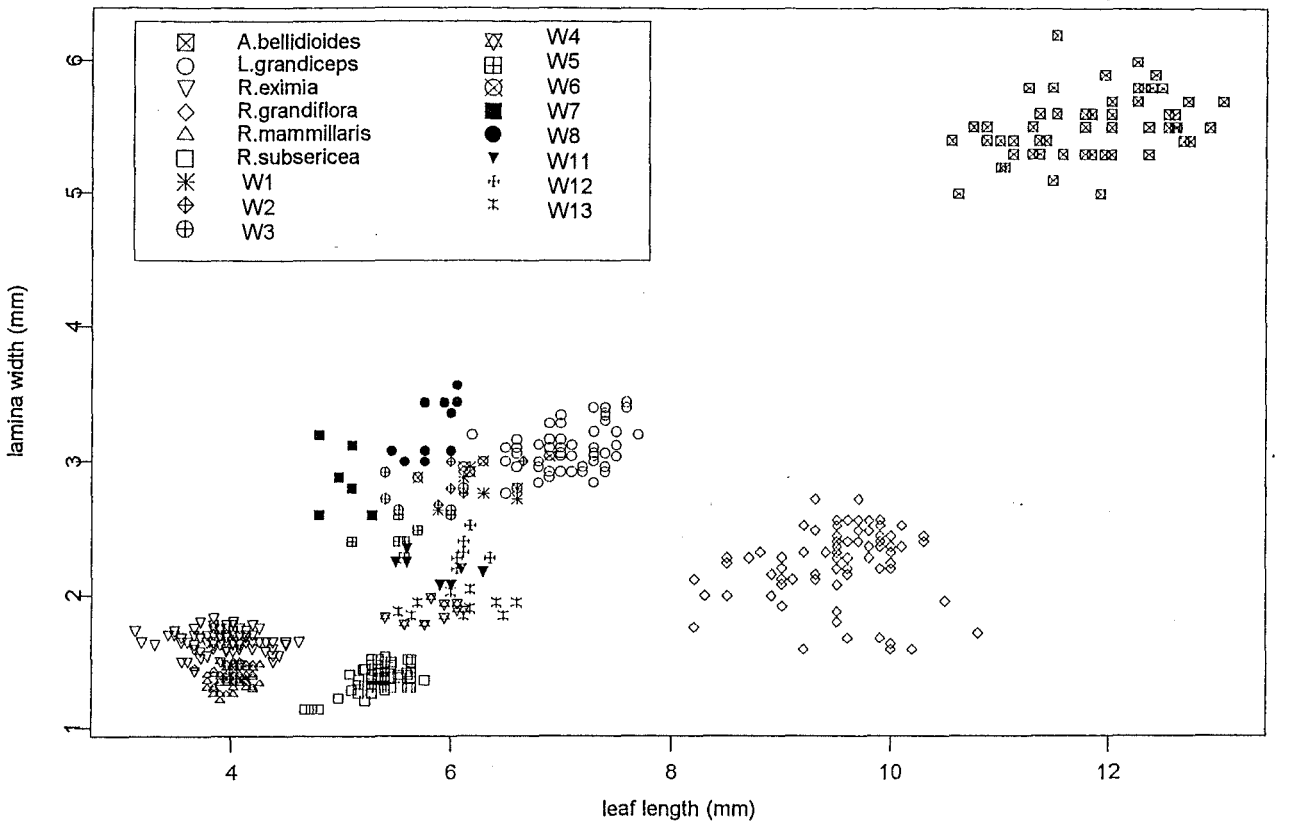


Figure 5.2. Leaf length and maximum lamina width in the putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and all sympatric gnaphalioid species.

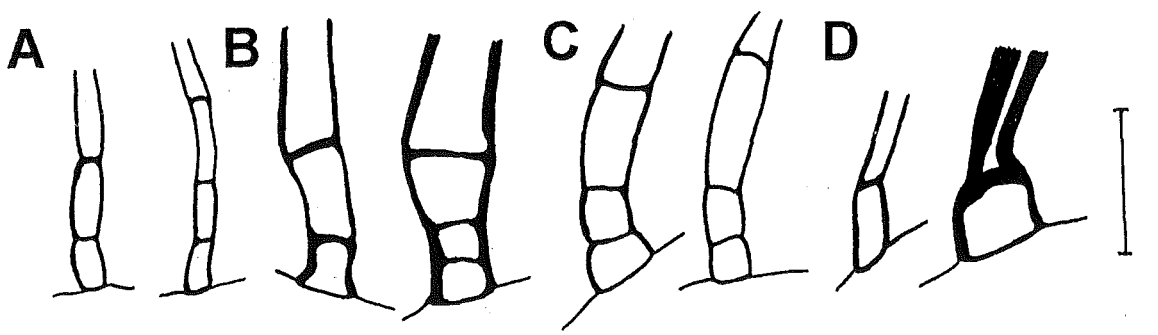


Figure 5.3. Clothing trichome basal cells from *Leucogenes grandiceps*, *Raoulia eximia*, *R. mammillaris* and a putative hybrid between *Leucogenes grandiceps* and *Raoulia eximia*. **A**, *L. grandiceps*; **B**, *R. eximia*; **C**, *W11*; **D**, *R. mammillaris*. Scale = 10 μm.

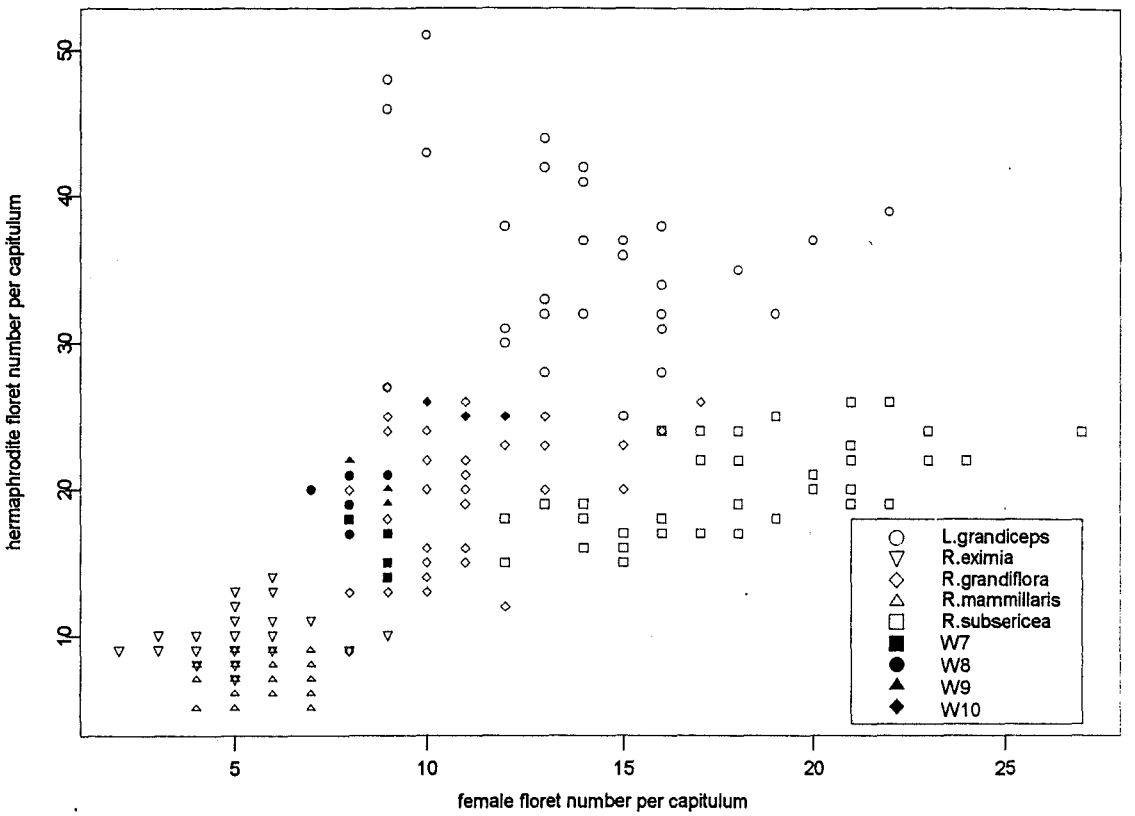


Figure 5.4. Female and hermaphrodite floret number per capitulum in the putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and all sympatric gnaphalioid species except *Anaphalioides bellidioides*.

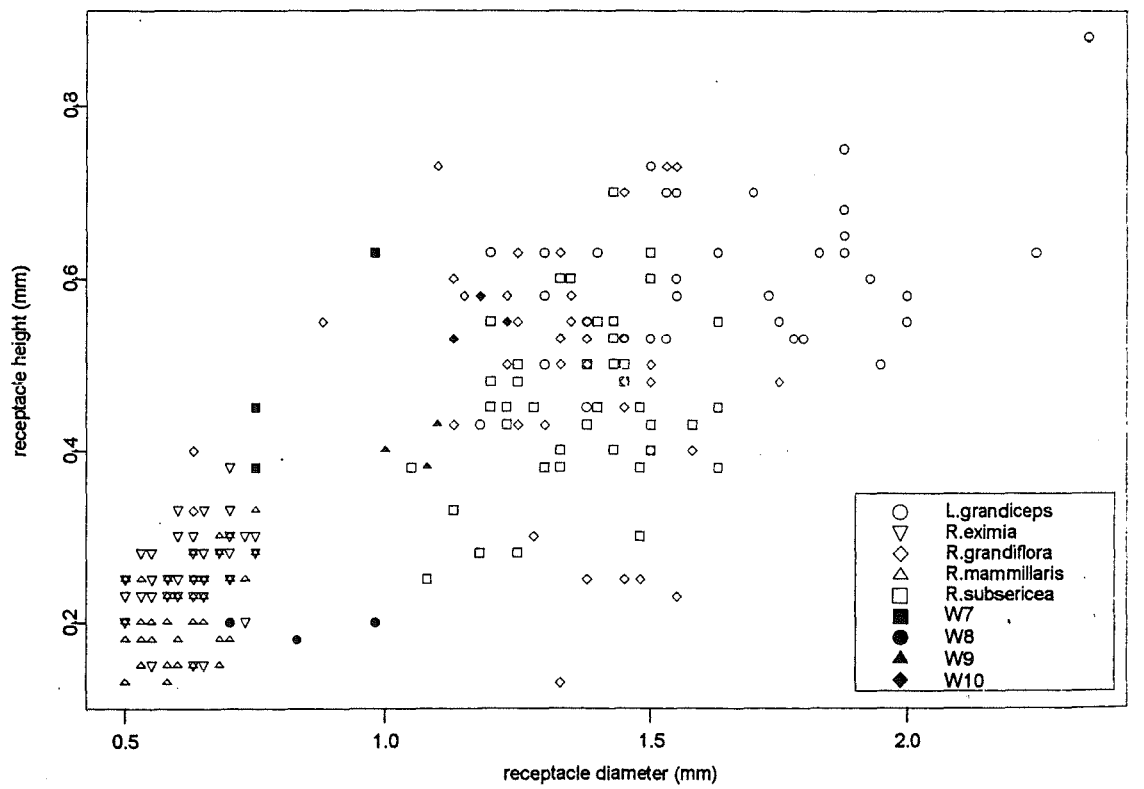


Figure 5.5. Receptacle diameter and height in the putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and all sympatric gnaphalioid species except *Anaphalioides bellidioides*.

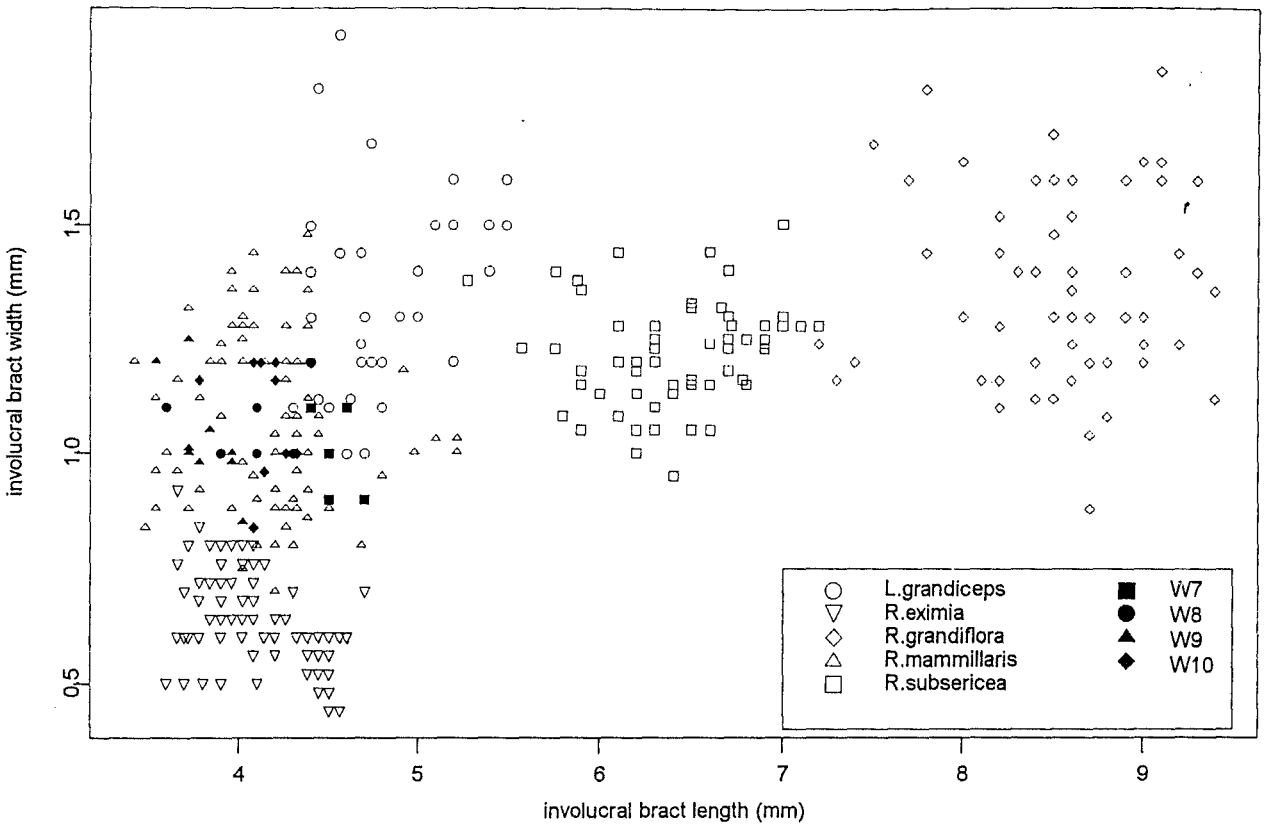


Figure 5.6. Inner involucral bract length and maximum width in the putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and all sympatric gnaphalioid species except *Anaphalioides bellidioides*.

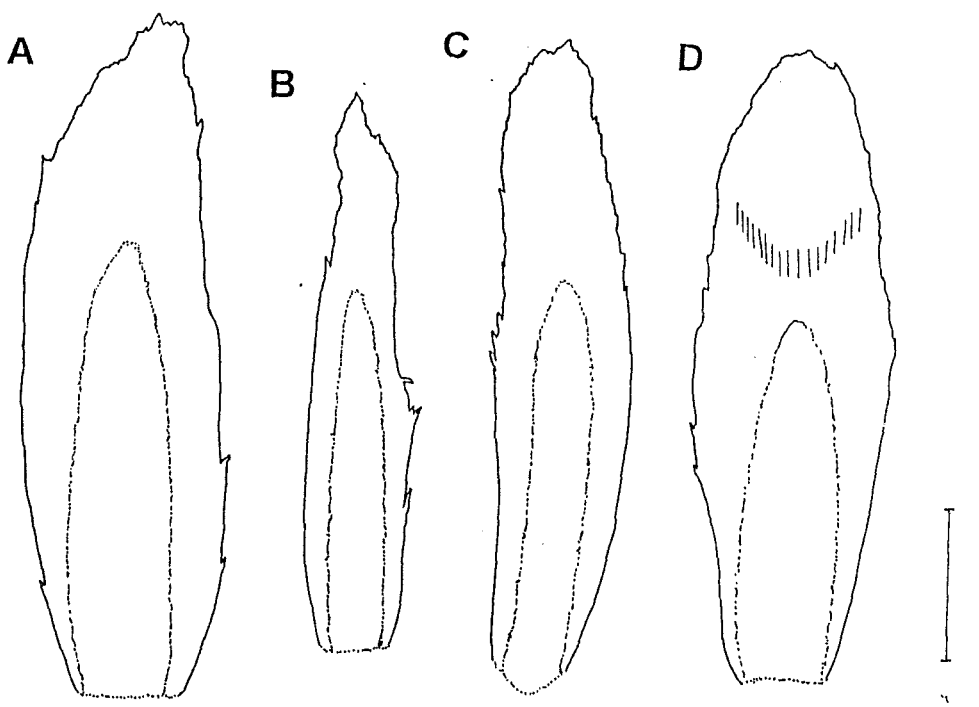


Figure 5.7. Inner involucral bracts of a *Leucogenes grandiceps*, *Raoulia eximia*, *R. mammillaris* and a putative hybrid between *L. grandiceps* and *R. eximia*. A, *L. grandiceps*; B, *R. eximia*; C, *W8*; D, *R. mammillaris*. Scale = 1 mm.

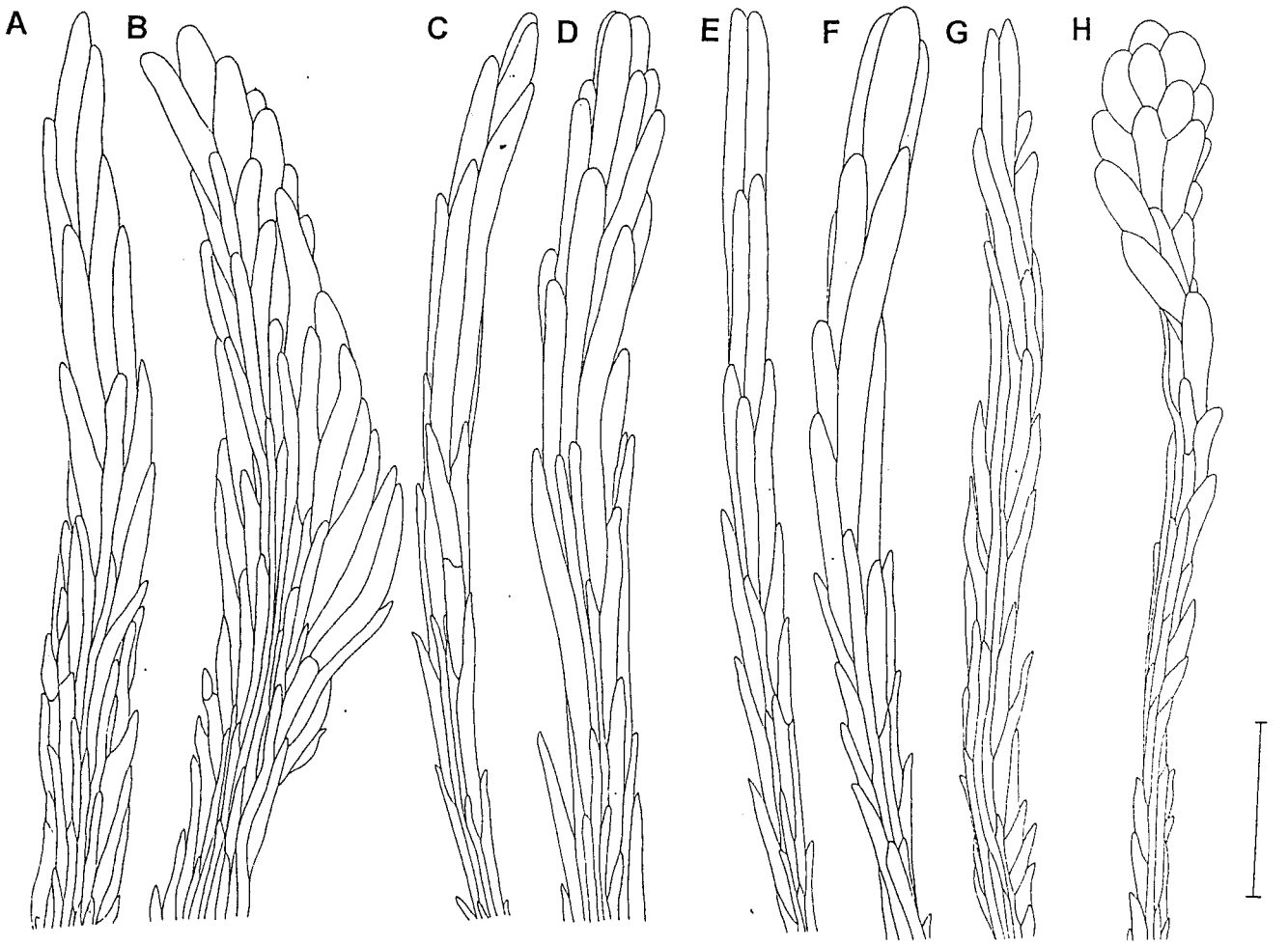


Figure 5.8. Pappus hair apices from *Leucogenes grandiceps*, *Raoulia eximia*, *R. mammillaris* and a putative hybrid between *L. grandiceps* and *R. eximia*.

A, *R. eximia* pappus hair from a female floret.

B, *R. eximia* pappus hair from a hermaphrodite floret.

C, *L. grandiceps* pappus hair from a female floret.

D, *L. grandiceps* pappus hair from a hermaphrodite floret.

E, *W7* pappus hair from a female floret.

F, *W7* pappus hair from a hermaphrodite floret.

G, *R. mammillaris* pappus hair from a female floret.

H, *R. mammillaris* pappus hair from a hermaphrodite floret.

Scale = 0.2 mm.

5.3.4 Analyses of morphological data

Description of analyses to identify the most likely parental species (i.e., all sympatric species were included in the data set) and to characterise the putative hybrids (i. e., only the putative hybrids and the most likely parental species were included in the data set) are combined in each of the following subsections. Support for which species to include in the character counts and character indices was obtained from the other analytic methods, but for consistency the organisation of this section is identical to that used in Chapter 4. For character codes, see Table 5.5–Table 5.8 (following pp. 211–214) and Appendix 5. The availability of vegetative and floral characters for each putative hybrid is summarised in Table 5.1 (p. 195).

Character count

The method of determining the parental intervals for continuous characters had only a minor impact on the classification of character states (Table 5.9 & Table 5.10 p. 233). The frequency of intermediate and extreme characters was slightly higher when quartile intervals were employed.

In *W7*, *W8*, *W9* and *W10* continuous characters were classified predominantly as intermediate. Up to one-third of continuous characters were classifed as parental or extreme, and three-quarters of discrete characters were classified as parental. The relative frequency of *L. grandiceps* and *R. eximia* characters varied, but *R. eximia* characters predominated (60–75 %). In most putative hybrids for which only vegetative characters were accessible, intermediate and parental characters were present in similar frequencies, but *L. grandiceps* characters predominated in *W1* and *W5*. In this group discrete characters were predominantly classified as parental. Most continuous characters were classified as intermediate, but only five such characters were recorded in these putative hybrids. Extreme characters were rare, occurring in four putative hybrids (*W6*, *W7*, *W8* and *W13*) and in the three continuous characters of lamina width, receptacle height, and corolla-tube length of female florets. *W13* was unique in possessing predominantly one basal cell in the clothing trichomes; this character was therefore only included in the character count (as an extreme character) for *W13*.

Character index

In all indices the putative hybrids were intermediate between the putative parental species and C-values were more variable among the putative hybrids than among the two species (Figure 5.9 p. 234). The characters used to derive the indices had a notable impact on C-values.

Among continuous characters, floral characters exhibited greater variation among plants of both putative parental species than did vegetative characters.

C-values derived from mixed characters were 0.83–0.91 among plants of *L. grandiceps*, 0.11 or less in *R. eximia*, and among the putative hybrids ranged from 0.42 in *W9* to 0.68 in *W5* and 0.69 in *W1*. C-values between 0.57 and 0.61 were recorded for *W2*, *W3*, *W4* and *W12*. Of the four putative hybrids for which floral characters were accessible (*W7*, *W8*, *W9* and *W10*), C-values ranged from 0.42 to 0.49. Inclusion of six continuous floral characters (50, 55, 65, 73, 88 and 90) with overlapping ranges in the parental means resulted in slightly reduced C-values for plants of *L. grandiceps* and slightly raised C-values for *R. eximia* plants. However, these characters had a negligible impact on C-values for *W7*, *W8*, *W9* and *W10*. Greater variation in continuous characters than in discrete characters was evident among plants of *L. grandiceps*, in which C-values derived from continuous characters were lower and more variable, but C-values were little different among plants of *R. eximia*. Among the putative hybrids, C-values derived from continuous characters were notably higher than for discrete characters in *W1*, *W6* and *W12*, lower in *W5*, *W8*, *W10* and *W11*, and little different in the other putative hybrids. For *W8* and some *L. grandiceps* plants, C-values derived from continuous floral characters were lower than for discrete floral characters, particularly when the continuous characters with overlapping parental mean ranges were included, but there was little difference in plants of *R. eximia*, *W7*, *W9* and *W10*. C-values derived from discrete characters exceeded those derived from continuous characters for *W5*, *W10* and *W11*, but the reverse was true for *W1*, *W6* and *W12*. The three character indices placed *W13* (C-values 0.54 and 0.55) among the other putative hybrids and almost equidistant between *L. grandiceps* and *R. eximia*.

Both species exhibited little variation in C-values derived solely from vegetative characters, whereas C-values for putative hybrids ranged from 0.41 for *W9* to 0.69 for *W1*. Compared to vegetative characters, C-values derived from floral characters were slightly lower for *L. grandiceps* plants and marginally higher for *R. eximia* plants. Of the putative hybrids for which capitula were available for study, C-values derived from vegetative and floral characters were very similar for *W9* and *W10*, but in *W7* and *W8* C-values derived from floral characters were markedly lower than for vegetative characters.

Canonical discriminant analysis

In all analyses, Box's M and adjusted M tests indicated high homogeneity of covariances ($P > 0.999$). Wilk's lambda, Pillai's trace, the Hotelling-Lawley trace and Roy's greatest root (all $P = 0$) indicated the difference in equality of the group means was highly significant. Hotelling's T^2 test indicated the differences between group means were highly significant (all $P < 0.001$). The canonical variates were not significantly correlated (all $P = 0$). The rule mean squared error was zero. All OTUs of the species were correctly classified by plug-in classification and cross-validation. For the putative hybrids the probability of group membership predicted by plug-in classification was always $P = 1$.

When all available characters were included in the analysis, the first, second and third canonical variates explained 82.7 %, 10.3 % and 3.6 % of the total variation. In scatter plots of the canonical variates, each species formed a very tight, well dispersed group (Figure 5.10 p. 235). *W7* was intermediate between *L. grandiceps* and *R. eximia* on each axis, but *W8* was never intermediate in the scatterplots. *W8* was placed with *R. eximia* on the first canonical variate, *L. grandiceps* on the second axis and was more extreme than *R. eximia* on the third canonical variate. Many characters were strongly correlated with the first canonical variate (including floret numbers per capitulum, capitulum width, receptacle dimensions and mucro length) or contributed similarly to the first and second axes (Figure 5.11 A p. 236). The character rankings (based on the absolute Pearson's product-moment correlation coefficients) for the first three canonical variates are summarised in Table 5.11 (p. 237). Plug-in classification predicted *W7* belonged to *L. grandiceps* and *W8* to *R. eximia*.

After characters with outliers were excluded, the first, second and third canonical variates accounted for 70.1 %, 14.8 % and 9.2 % of the total variation. *W8* was intermediate between *L. grandiceps* and *R. eximia* on the first and second axes but *W7* was intermediate on the first axis only (Figure 5.12. p. 238). Both putative hybrids were more extreme than, but close to, *R. eximia* on the third canonical variate. Most characters were strongly correlated with the first canonical variate, but only leaf type A glandular trichome dimensions and the female:hermaphrodite floret ratio strongly contributed to the second canonical variate (Figure 5.11 B p. 236). Plug-in classification predicted both putative hybrids belonged to *L. grandiceps*.

Four vegetative characters (leaf length, lamina width, leaf length:lamina width ratio and mucro length) were excluded from the data set to enable a simultaneous comparison of *W7*,

W8, *W9* and *W10* with the sympatric species. The canonical variates explained 45.6 %, 32.2 % and 9.8 % of the total variation respectively. The species again formed tight, well-dispersed clusters (Figure 5.13 p. 239). The putative hybrids did not form a distinct group on the scatter plots but were intermediate between or close to *L. grandiceps* and *R. eximia* on each axis. *W7* was intermediate between the putative parents on the first and third canonical variates, but slightly more extreme than *R. eximia* on the second axis. *W8* was closer to *R. eximia* on the first and second axes but clustered with *L. grandiceps* on the third canonical variate. *W9* and *W10* were close to *L. grandiceps* on the first axis and close to *R. eximia* on the second and third canonical variates. Few characters were strongly correlated with the first and second canonical variates (Figure 5.14 A p. 240). Clothing trichome basal cell dimensions and the pappus-hair apical cell number in hermaphrodite florets were the greatest contributors to the first axis; leaf type A glanduar trichome length, female floret number per capitulum and female:hermaphrodite floret ratio were most important on the second canonical variate. Plug-in classification predicted *W7* and *W8* belonged to *R. eximia*, and *W9* and *W10* to *L. grandiceps*.

After characters containing outliers were excluded, the first three canonical variates accounted for 55.7 %, 28 % and 6.9 % of the total variation. *W7* was intermediate between *L. grandiceps* and *R. eximia* on the first and second canonical variates, but clustered with *L. grandiceps* on the third axis (Figure 5.15 p. 241). *W8* was intermediate between the putative parents on all axes. *W9* and *W10* were close on each canonical variate; the two plants were intermediate on the first and second axes but clustered with *R. eximia* and *R. subsericea* on the third canonical variate. The characters contributing most to the canonical variates differed from the previous analysis. Numerous characters were strongly correlated with the first canonical variate, but only leaf type A glandular trichome width was highly correlated with the second axis (Figure 5.14 B p. 240). Plug-in classification predicted all four putative hybrids belonged to *L. grandiceps*.

Floral characters were excluded to enable comparison of all putative hybrids except *W9* and *W10* with the sympatric species. The canonical variates explained 78.8 %, 14.1 % and 5.5 % of the variation. Overall, discrimination of the species (particularly *R. eximia* and *R. mammillaris*) was reduced. The first canonical variate principally discriminated *A. bellidioides*, *R. grandiflora* was distinguished on the second canonical variate and *R. subsericea* was isolated on the third axis (Figure 5.16 p. 242). Relationships among the other plants were not clearly resolved. Leaf length, leaf type A glandular trichome length and leaf

length:width ratio were highly correlated with the first, second and third canonical variates respectively. Plug-in classification predicted *W5* belonged to *R. subsericea*, *W8* to *R. grandiflora* and *W11* to *R. eximia*. The other putative hybrids were predicted to belong to *L. grandiceps*. Exclusion of mucro length (which contained outliers) from the data set had little impact on the canonical variates. However, group membership predictions differed. *W5* was predicted to belong to *R. eximia*, whereas all other putative hybrids were predicted to belong to *L. grandiceps*.

Exclusion of *A. bellidioides*, *R. grandiflora* and *R. subsericea* improved discrimination of *R. eximia* and *R. mammillaris* on the second canonical variate (Figure 5.17 p. 243). With mucro length included in the data set, the first canonical variate accounted for 93.8 % of the variation and principally discriminated *L. grandiceps* from the other plants. The putative hybrids were placed close to *R. eximia* or *R. mammillaris* on both axes and intermediacy with *L. grandiceps* was not suggested. On the second canonical variate *W5*, *W6*, *W7* and *W8* were more extreme than *R. eximia*, whereas *W4* and *W13* were close to *R. mammillaris*. The other putative hybrids clustered with *R. eximia*. Exclusion of mucro length from the data set improved discrimination among the putative hybrids on both axes and resulted in their placement closer to *L. grandiceps*. Intermediacy between *L. grandiceps* and *R. eximia* was suggested for most putative hybrids except *W4*, *W12* and *W13*, which were more extreme. The first canonical variate explained 90.4 % of the variation. Leaf dimensions and the basal cell length and terminal cell width of the clothing trichomes were highly correlated with the first canonical variate. Leaf length:width ratio and clothing trichome basal cell width were the greatest contributors to the second canonical variate.

Cluster analysis

Agglomerative hierarchical clustering with group-average linkage was performed on dissimilarities calculated from the complete data set. The six species were clearly differentiated and the putative hybrids formed a single cluster linked to *L. grandiceps*, which was in turn linked to *R. eximia* cluster (Figure 5.18 p. 244). Each species cluster exhibited relatively little variation among OTUs. The maximum dissimilarity within any species clusters was 0.055, whereas the putative hybrids, with a greatest dissimilarity of 0.11, were only slightly more variable than the species. *W3* and *W7* were the most similar putative hybrids, while *W1* and *W5* were the most dissimilar to the other putative hybrids. *W13* was placed within the putative-hybrid cluster. The overall Pearson's product-moment and Spearman's rank correlation coefficients for the phenogram was 0.92 and 0.90 respectively,

and the lowest coefficients for individual linkages were 0.79 (the linkage between the *W3/W4/W7* and *W6/W8/W10/W11* clusters) and 0.65 (two linkages within the *R. grandiflora* cluster) respectively (Figure 5.19 p. 245). Six randomisations of the OTU order in the data set returned 33 variant phenograms. Linkage of *W1*, *W5* and *W13* was unchanged, but clustering of the other putative hybrids varied among the alternative phenograms.

Exclusion of *A. bellidioides*, *R. grandiflora* and *R. subsericea* from the data set altered the hierarchy of the phenogram. Two major clusters (*L. grandiceps*/putative hybrids and *R. eximia/R. mammillaris*) were formed and overall the linkages were at higher dissimilarities; e.g., in the group-average-linkage phenogram the highest dissimilarity in the putative-hybrid cluster was 0.22 (Figure 5.20 p. 246). Clustering patterns among the putative hybrids also differed slightly. The clustering of *W1* with *W5*, and of *W3*, *W4* and *W7*, was consistent in the group-average-linkage phenograms produced from the two data sets, but *W13* was now the most dissimilar putative hybrid. Most of the putative hybrids formed two major clusters, but correlation coefficients for the lower linkages within each cluster were among the lowest in the phenogram. *W1*, *W5* and *W13* were the most dissimilar of the putative hybrids. Compared to analyses of the complete data set, the overall Spearman's rank correlation coefficients were notably lower (the coefficient for the group-average-linkage phenogram was 0.75). In contrast, the overall Pearson's correlation coefficients were more similar to those of analyses of the complete data set (e.g., the coefficient for the group-average-linkage phenogram was 0.89) and less variable between linkage methods, but coefficients for some linkages were lower (Figure 5.23 p. 251).

HYWIN

Each analysis of the complete data set generated 97 527 hypotheses. The 410 highest-ranked combinations represented the 0.95 probability that all specimens would be ranked as a hybrid. Plants of the sympatric species, particularly *L. grandiceps* and *R. grandiflora*, rather than the putative hybrids were often suggested to be hybrids and only with mixed data and a low parental-distance weighting were *L. grandiceps* × *R. eximia* hypotheses highly ranked. The putative hybrids were most commonly ranked as *A. bellidioides* × *R. eximia* and were occasionally suggested to be a parent.

Analyses of the data set following exclusion of *A. bellidioides*, *R. grandiflora* and *R. subsericea* generated 23 310 hypotheses. The 246 highest-ranked hypotheses represented the 0.95 probability that all specimens would be ranked as a hybrid. Plants of *L. grandiceps*, *R.*

eximia and *R. mammillaris* were rarely suggested to be hybrids. The putative hybrids and parentage hypotheses receiving high rankings varied with the weightings employed (see Table 5.12 p. 248).

In all analyses of continuous characters *L. grandiceps* × *R. mammillaris* hypotheses predominated among the highest-ranked hypotheses. The default weightings (wI = 1, wE = 1, wP = 1) resulted in all putative hybrids except *W5* being suggested to be hybrids. Only three putative hybrids (*W2*, *W3* and *W7*) were not suggested to be parents. *W7*, *W8*, *W9* and *W10* were occasionally suggested to be *L. grandiceps* × *R. eximia*. Reducing the intermediacy weighting (wI = 0.1, wE = 1, wP = 1) resulted in the ranking of only seven putative hybrids (*W4*, *W7*, *W8*, *W9*, *W10*, *W11* and *W13*) as hybrids. *W1* and *W5* were commonly ranked as parents with a plant of *R. eximia* or *R. mammillaris* always the other parent. *W7*, *W8*, *W9*, *W10* and *W13* were suggested to be *L. grandiceps* × *R. eximia*. With reduced intermediacy and equality weightings (wI = 0.1, wE = 0.1, wP = 1), all putative hybrids were suggested to be hybrids and only *W1* was suggested to be a parent. The same putative hybrids as in the previous analysis, with the addition of *W11*, were suggested to be *L. grandiceps* × *R. eximia*.

In all analyses of mixed data, predominantly *L. grandiceps* × *R. eximia* hypotheses were highly ranked. With the default weightings all putative hybrids except *W1*, *W5* and *W13* were ranked as hybrids. *W7*, *W8* and *W9* were least frequently ranked as hybrids. *W1* was ranked once as a parent of *W11* (with *R. eximia*). *W4* was, in some hypotheses, suggested to be *L. grandiceps* × *R. mammillaris*. A low parental-distance weighting (wI = 1, wE = 0.1, wP = 0.1) produced a very similar outcome, but *L. grandiceps* × *R. mammillaris* hypotheses were more predominant. A low equality weighting (wI = 1, wE = 0.1, wP = 1) also produced a similar outcome to the default weightings, but *W1* was occasionally suggested to be a parent of *W3* and *W7* (with *R. eximia*) rather than *W11*. When intermediacy received a low weighting (wI = 0.1, wE = 1, wP = 1), *L. grandiceps* × *R. eximia* hypotheses filled the 51 highest rankings (Table 5.13 p. 249). All putative hybrids except *W1*, *W2*, *W3* and *W5* were suggested to be hybrids, and both *W1* and *W5* were suggested twice to be a parent of *W11* (with *R. eximia*). *L. grandiceps* × *R. mammillaris* hypotheses were less frequently ranked.

Multidimensional scaling

In an analysis of the complete data set, the species were well differentiated and formed tight clusters on scatterplots of the first, second and third principal coordinates (Figure 5.24 A–C

p. 252). These axes explained 51.1 %, 16.4 % and 9.1 % of the total variation respectively and the stress values were 0.1491, 0.0610 and 0.0342 respectively. Each species was clearly discriminated on the first axis and the putative hybrids were, together with *R. mammillaris*, intermediate between *L. grandiceps* and *R. eximia*. The second principal coordinate largely separated *A. bellidioides*, *R. grandiflora* and *R. subsericea*, and the putative hybrids were split into two distinct groups: those for which floral characters were available clustered with *R. mammillaris*, and those for which only vegetative characters were available were placed closer to *R. grandiflora* and *R. subsericea*. *Raoulia mammillaris* and *L. grandiceps* were isolated on the third axis but the other species were less clearly separated. The putative hybrids for which floral characters were available (*W7*, *W8*, *W9* and *W10*) were placed between *L. grandiceps* and *R. eximia*, but the other putative hybrids were intermediate between *L. grandiceps* and *R. grandiflora*. *W13* was more extreme than *R. eximia* and the other putative hybrids, and was placed closer to *R. subsericea*.

In a second analysis, the least likely parental species (*A. bellidioides*, *R. grandiflora* and *R. subsericea*) were excluded from the data set (Figure 5.23 A–C p. 251). The first, second and third axes accounted for 47.8 %, 27.9 % and 6.0 % of the total variation respectively. Compared to the complete data set, the stress value for the first axis was higher (0.2673) but markedly lower for the second and third axes. *Leucogenes grandiceps*, *R. eximia* and *R. mammillaris* were well separated on scatterplots of the first and second principal coordinates (Fig. 5.23 A–C p. 267). Most putative hybrids were placed intermediate between *L. grandiceps* and *R. eximia*, but *W1* and *W5* were placed close to *L. grandiceps* and *W13* was intermediate between *L. grandiceps* and *R. mammillaris*. The putative hybrids for which floral characters were available were the most similar to *R. eximia*. Variation among the putative hybrids was much greater than within the species clusters. The third axis principally separated specimens within groups and the putative hybrids were placed at one extreme; *W13* was notably isolated from all other specimens.

Split decomposition

Split-decomposition analysis with all sympatric species included identified 27 weakly compatible splits (Figure 5.24 A & B p. 252). The splits graph was reasonably well resolved and the fit was 73 %. The longest edge separated *A. bellidioides* from the other species and the putative hybrids. A contradictory edge connected *A. bellidioides* and *L. grandiceps*. A split separated *A. bellidioides*, *R. grandiflora* and *R. subsericea* from the other species and putative hybrids. The putative hybrids formed a single group, lacking a basal edge, placed

between *L. grandiceps* and the *R. eximia* and *R. mammillaris* splits. A contradictory split linked *W12* and *R. grandiflora*. A short edge united *W3* and *W7*, and *W10* and *W11*, but relationships among the putative hybrids were otherwise poorly resolved. *W13* was placed among the other putative hybrids. A Buneman tree contained 21 compatible splits and had a fit of 62 %. A split separated *A. bellidioides*, *R. grandiflora* and *R. subsericea* from the remaining specimens. No compatible splits were present among *L. grandiceps*, *R. eximia*, *R. mammillaris* and the putative hybrids. *W12* was placed with the other putative hybrids and was not linked to *R. grandiflora* by a compatible split.

Following exclusion of *A. bellidioides*, *R. grandiflora* and *R. subsericea* from the data set, a splits graph with 27 weakly compatible splits and a fit of 75.4 % was generated (Figure 5.24 C & D). Overall the splits graph was well resolved but contradictory internal edges existed for splits involving *L. grandiceps*, *R. eximia*, *W1*, *W2*, *W5*, *W9* and *W11*. Long terminal edges characterised *L. grandiceps*, *R. eximia* and *R. mammillaris*, and the putative hybrids did not form a single group. A split separated *W1* and *W5* from the other putative hybrids and connected these plants to *L. grandiceps*. There was some support for a similarity between *W9*, *W11* and *R. eximia*. A short split united *W3* and *W7* but relationships among the putative hybrids were poorly resolved. A weakly supported edge linked *W13* and *R. mammillaris*. The terminal edges were variable in length among the putative hybrids. A Buneman tree comprised 17 compatible splits and had a fit of 59.6 %. The only internal edge linked *R. eximia* and *R. mammillaris*. *L. grandiceps* and the putative hybrids produced a 'starburst' pattern.

Finally, dissimilarities among the putative hybrids were analysed separately. A splits graph containing 19 weakly compatible splits and with a fit of 71.2 % was generated (Figure 5.24 E & F). As indicated by the 'starburst' pattern of the splits graph, most relationships were poorly resolved. A split separated *W1* and *W5* from the other putative hybrids, and there was some support for the linkage of *W8*, *W10* and *W11*, and of *W2*, *W3*, *W7* and *W9*. A Buneman tree contained 14 compatible splits and had a fit of 67 %. The only internal edge separated *W1* and *W5* from the other putative hybrids.

Putative hybrid	Parental					Intermediate		Extreme	Novel
	L. grandiceps		*R. eximia*		Total				
	D	C	D	C		D	C	C	
W1	7	2	2	0	11	2	3	0	0
W2	4	1	3	0	8	4	4	0	0
W3	4	2	3	0	9	4	3	0	0
W4	4	2	3	0	9	4	3	0	0
W5	7	1	2	1	11	3	3	0	0
W6	3	2	4	0	9	4	3	0	0
W7	8	2	13	0	23	7	11	0	0
W8	8	1	13	1	23	7	9	2	0
W9	5	0	15	0	20	8	10	0	0
W10	8	3	12	0	23	8	7	0	0
W11	3	0	4	2	9	4	3	0	0
W12	4	1	4	0	9	3	4	0	0
W13	5	1	3	0	9	3	4	0	0

Table 5.9. Character counts for the putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* using standard-deviation intervals. Only characters discriminating the parental species were included. C, continuous characters; D, discrete characters.

Putative hybrid	Parental					Intermediate		Extreme	Novel
	L. grandiceps		*R. eximia*		Total				
	D	C	D	C		D	C	C	
W1	7	2	2	0	11	2	3	0	0
W2	4	1	3	0	8	4	4	0	0
W3	4	1	3	0	8	4	4	0	0
W4	4	2	3	0	9	4	3	0	0
W5	7	1	2	1	11	3	3	0	0
W6	3	1	4	0	8	4	3	1	0
W7	8	3	13	1	25	7	13	1	0
W8	8	0	13	3	24	7	12	3	0
W9	5	0	15	3	23	8	12	0	0
W10	8	1	12	3	24	8	11	0	0
W11	3	0	4	1	8	4	4	0	0
W12	4	1	4	0	9	3	4	0	0
W13	5	1	3	0	9	3	4	0	0

Table 5.10. Character counts for the putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* using quartile intervals. Only characters discriminating the parental species were included. C, continuous characters; D, discrete characters.

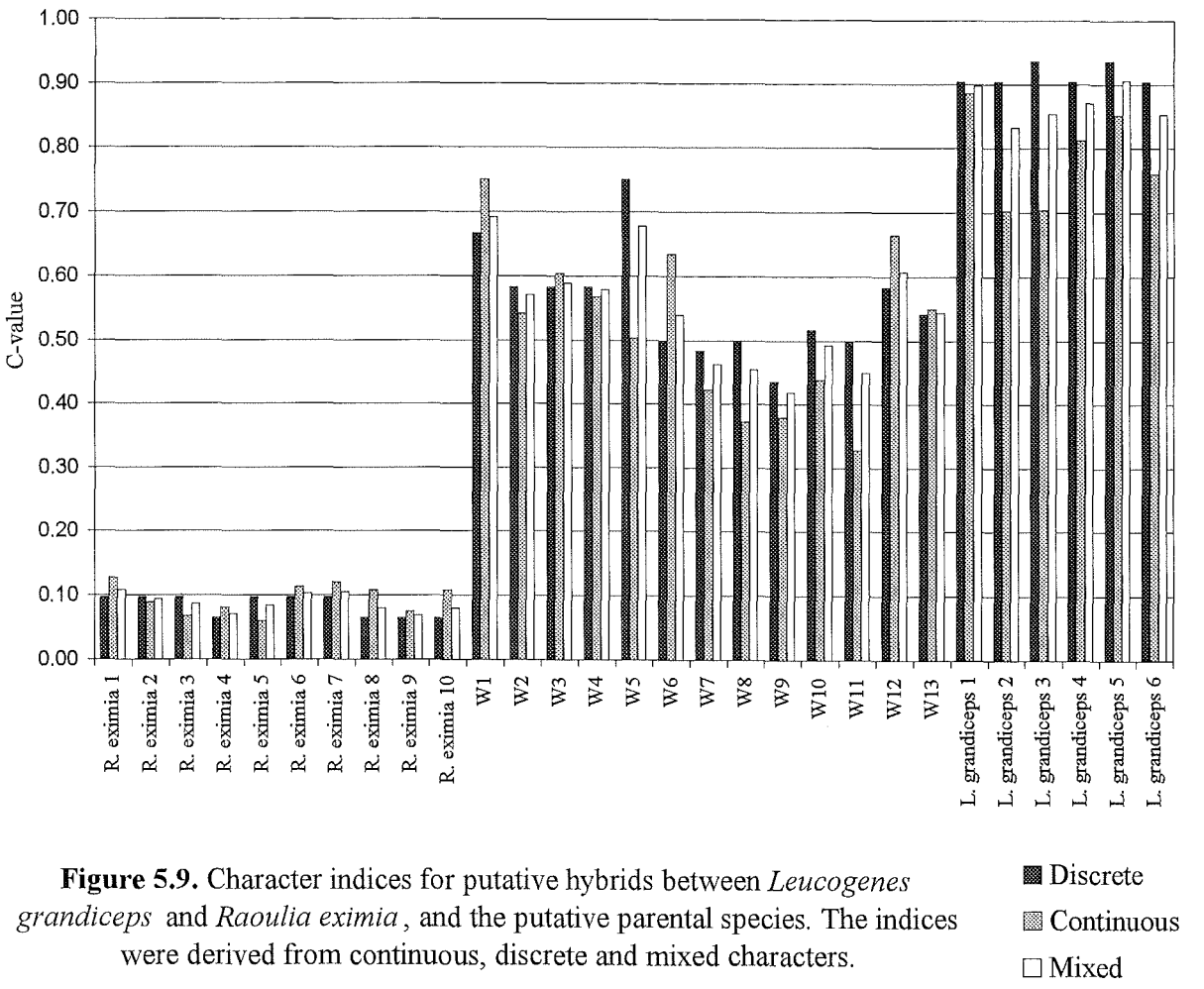


Figure 5.9. Character indices for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and the putative parental species. The indices were derived from continuous, discrete and mixed characters.

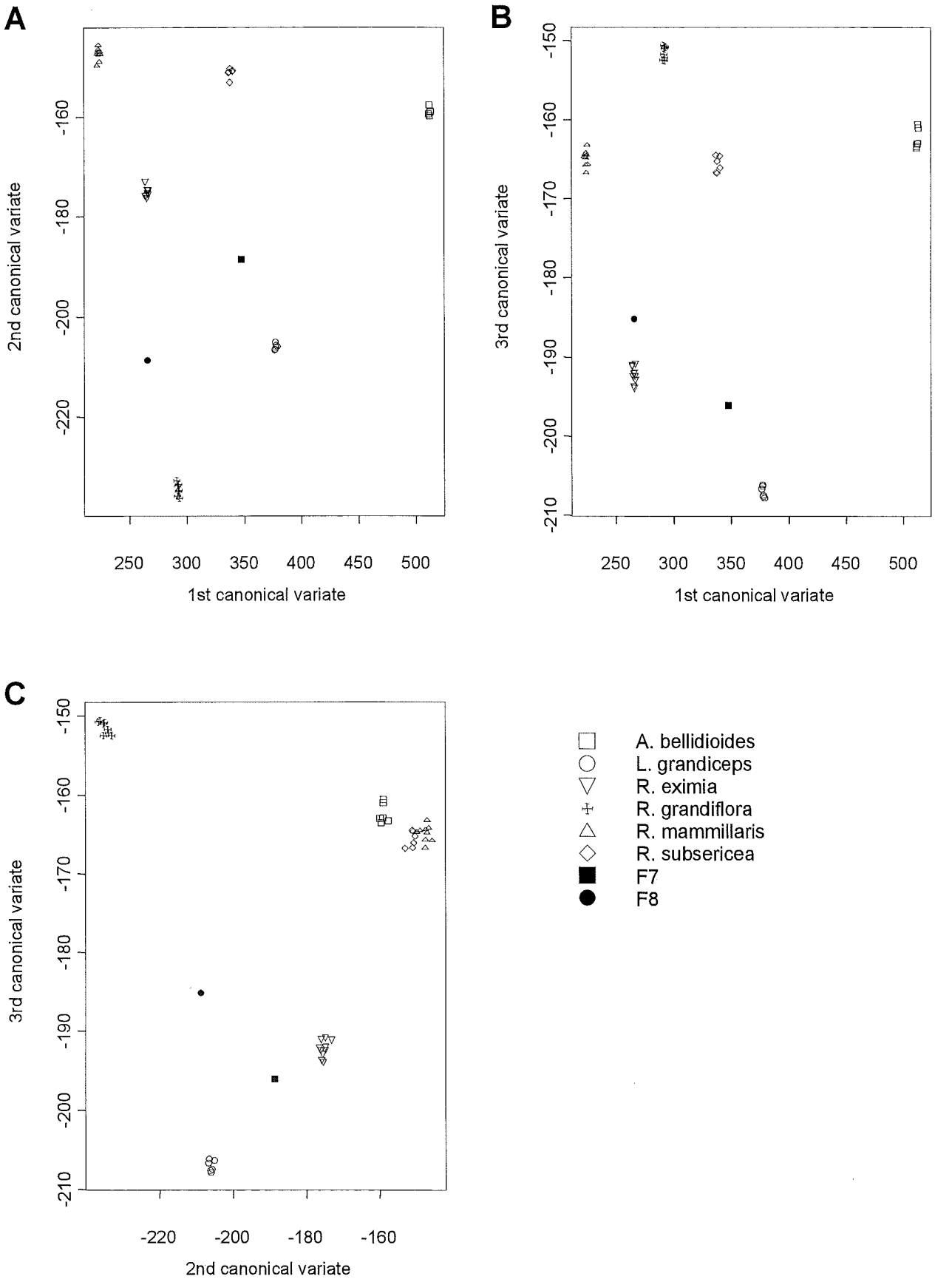


Figure 5.10. Scatter plots of the first, second and third canonical variates for two putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia (W7* and *W8*), and all sympatric gnaphalioid species. 36 continuous characters were analysed. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

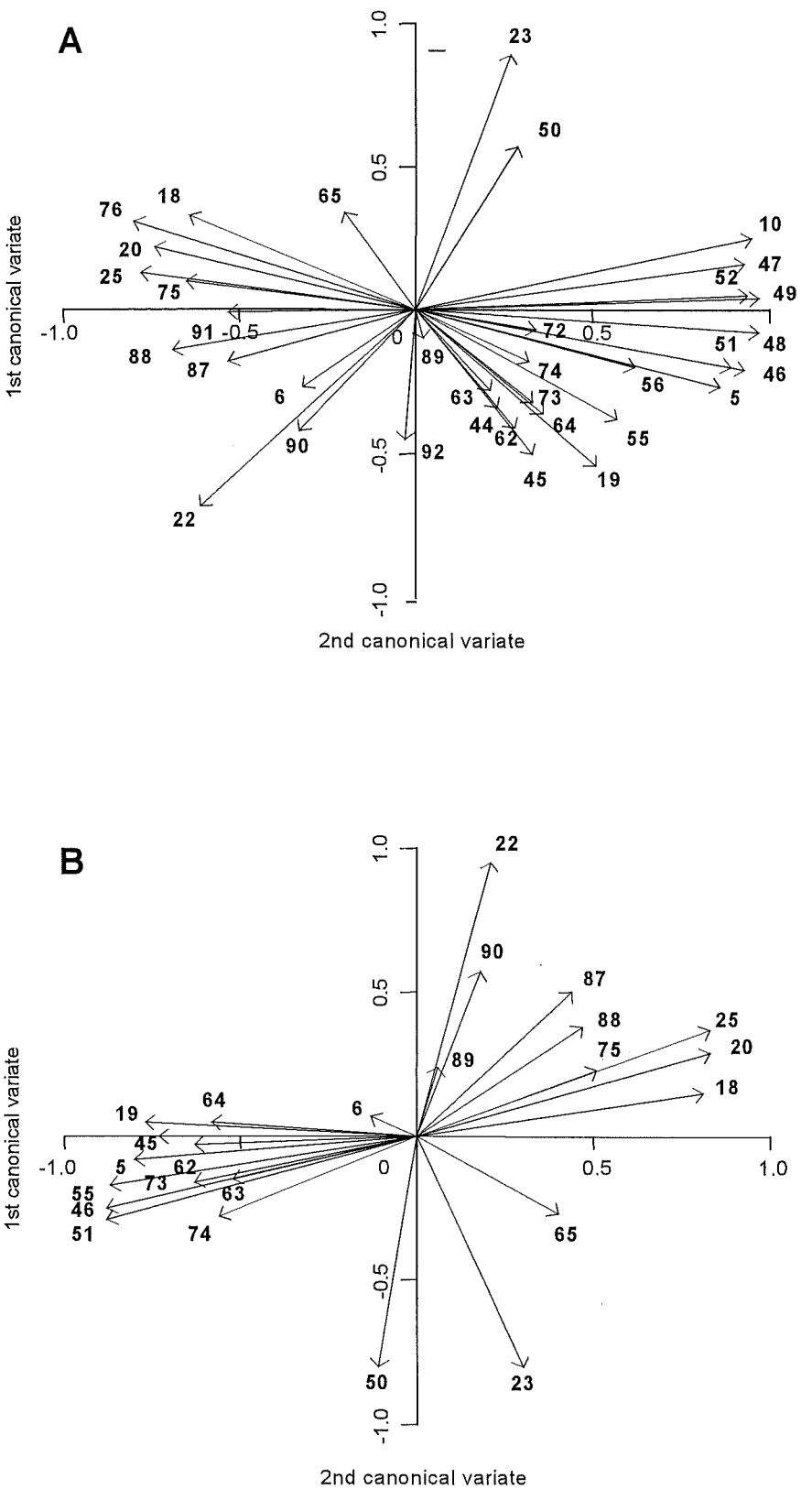


Figure 5.11. Vector plots of Pearson's product-moment correlation coefficients between the original data and the first and second canonical variates. **A**, Coefficients for an analysis of 36 continuous characters with all sympatric gnaphalioid species included (see Figure 5.10); **B**, coefficients for an analysis of 25 continuous characters with all sympatric gnaphalioid species included; characters containing outliers were excluded (see Figure 5.12).

Character	Canonical variate		
	First	Second	Third
Leaf length	0.7566	-0.4868	0.3898
Lamina width	0.8642	-0.2712	-0.0560
Leaf length: maximum lamina width ratio	-0.3243	-0.2705	0.6700
Mucro length	0.9496	0.2450	0.0987
Leaf type A glandular trichome length	-0.6130	-0.6752	-0.2845
Leaf type A glandular trichome maximum width	0.2730	0.8883	-0.0894
Type A glandular trichomes, terminal cell length	-0.7772	0.1254	-0.4977
Clothing trichome basal cell length	-0.6448	0.3293	-0.2481
Clothing trichome basal cell width	0.5126	-0.5430	0.3001
Clothing trichome terminal cell width	-0.7413	0.2168	-0.2942
Number of capitula per inflorescence	0.2342	-0.3409	-0.6716
Capitulum length	0.3339	-0.5025	0.6162
Capitulum width at midpoint	0.9254	-0.2086	0.1455
Number of female florets per capitulum	0.9276	0.1638	0.2734
Number of hermaphrodite florets per capitulum	0.9674	-0.0830	0.0628
Total number of florets per capitulum	0.9673	0.0355	0.1599
Female: hermaphrodite floret ratio	0.2890	0.5654	0.6169
Receptacle diameter	0.8950	-0.1977	0.1764
Receptacle height	0.9396	0.0472	0.2072
Inner involucral bract length	0.5730	-0.3843	0.6734
Inner involucral bract, maximum width	0.6231	-0.2001	0.4843
Corolla tube length in female florets	0.2795	-0.4083	0.5370
Corolla tube length in hermaphrodite florets	0.2058	-0.2782	0.5975
Hermaphrodite floret corolla tube, width at base of lobes	0.3603	-0.3644	0.1622
Hermaphrodite floret corolla tube, point of expansion from base:total length	-0.2016	0.3449	-0.1427
Style arm length in hermaphrodite florets	0.3435	-0.0720	-0.0153
Pappus hair length in female florets	0.3298	-0.3239	0.5989
Pappus hair length in hermaphrodite florets	0.3239	-0.1820	0.6115
Female-floret pappus hairs, number of apical cells	-0.6528	0.1014	0.0728
Hermaphrodite-floret pappus hairs, number of apical cells	-0.7983	0.3069	-0.1406
Female-floret ovary length	-0.5280	-0.1757	-0.3846
Female-floret ovary width	-0.6855	-0.1424	-0.0091
Female-floret ovary length:width ratio	0.0227	-0.0951	-0.4193
Hermaphrodite-floret ovary length	-0.3314	-0.4184	-0.3473
Hermaphrodite-floret ovary width	-0.5285	-0.0065	-0.0109
Hermaphrodite-floret ovary length:width ratio	-0.0281	-0.4537	-0.3454

Table 5.11. Ranking of characters (based on the absolute Pearson's product-moment correlation coefficients between the original data and the canonical coefficients) in the canonical discriminant analysis of 36 continuous characters (see Figures 5.10 and 5.11). fem. = female floret; herm. = hermaphrodite floret.

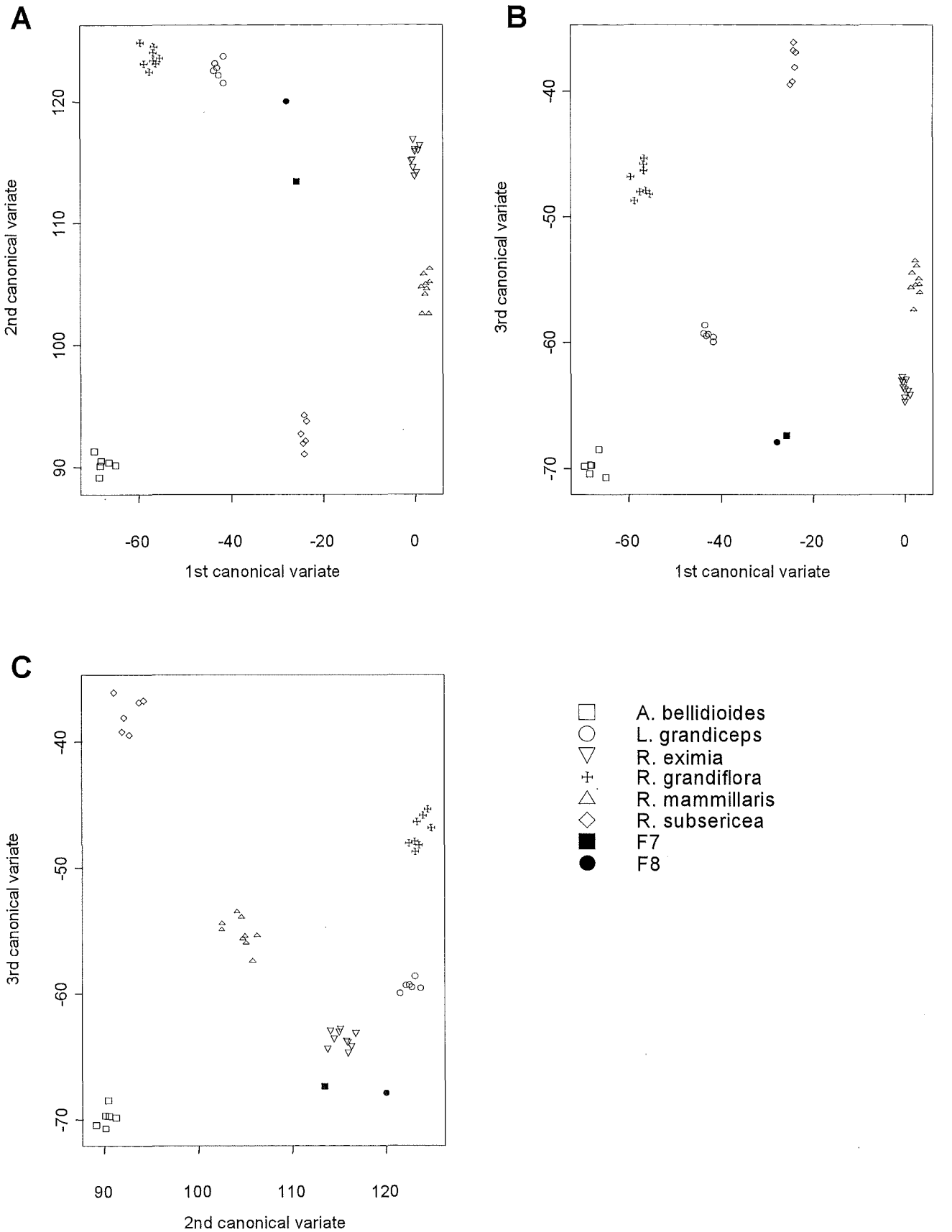


Figure 5.12. Scatter plots of the first three canonical variates for two putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* (*W7* and *W8*), and all sympatric gnaphalioid species. 25 continuous characters were analysed. Characters containing outliers were excluded. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

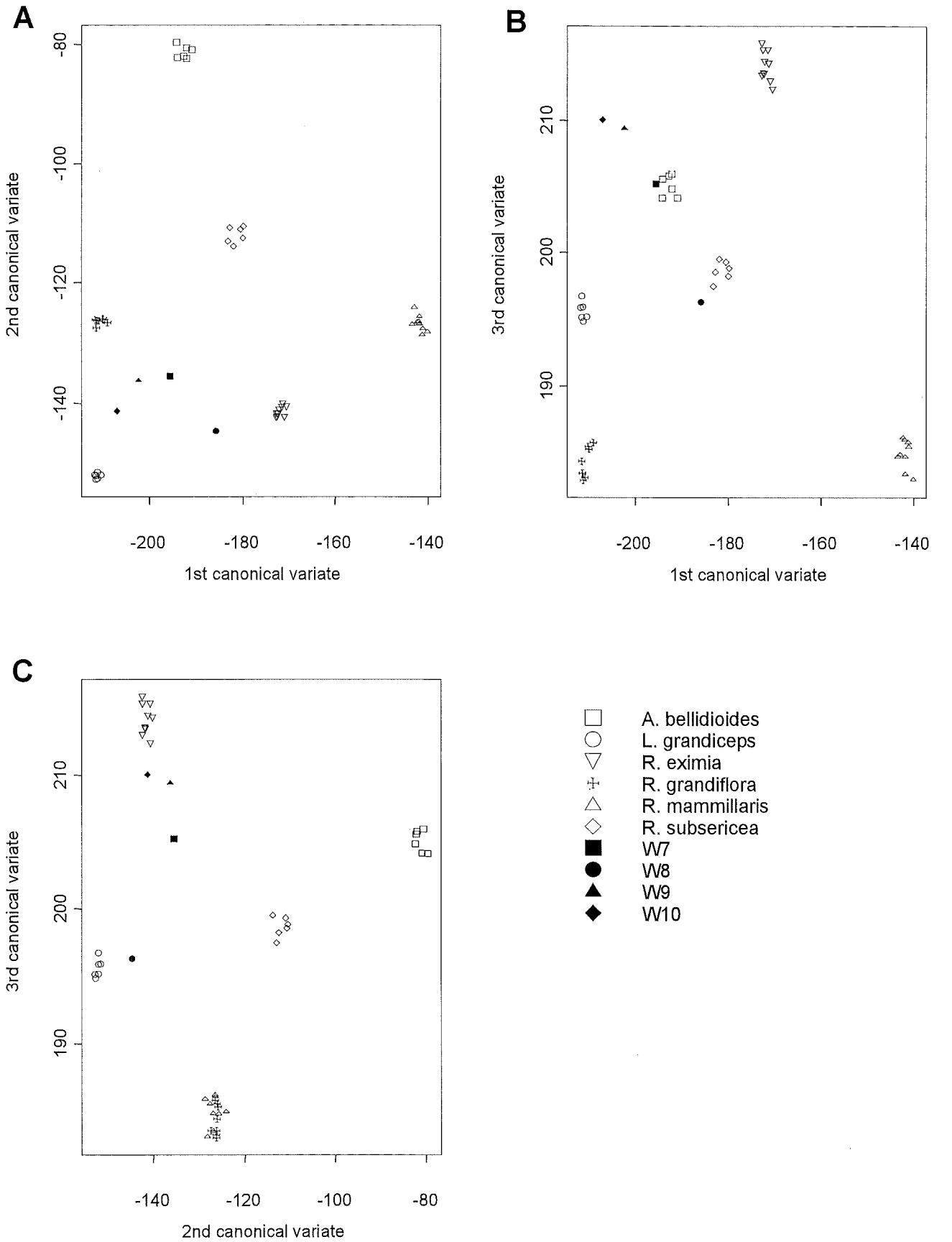


Figure 5.13. Scatter plots of the first three canonical variates for four putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* (*W7*, *W8*, *W9* and *W10*), and all sympatric gnaphalioid species. 32 continuous characters were analysed. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

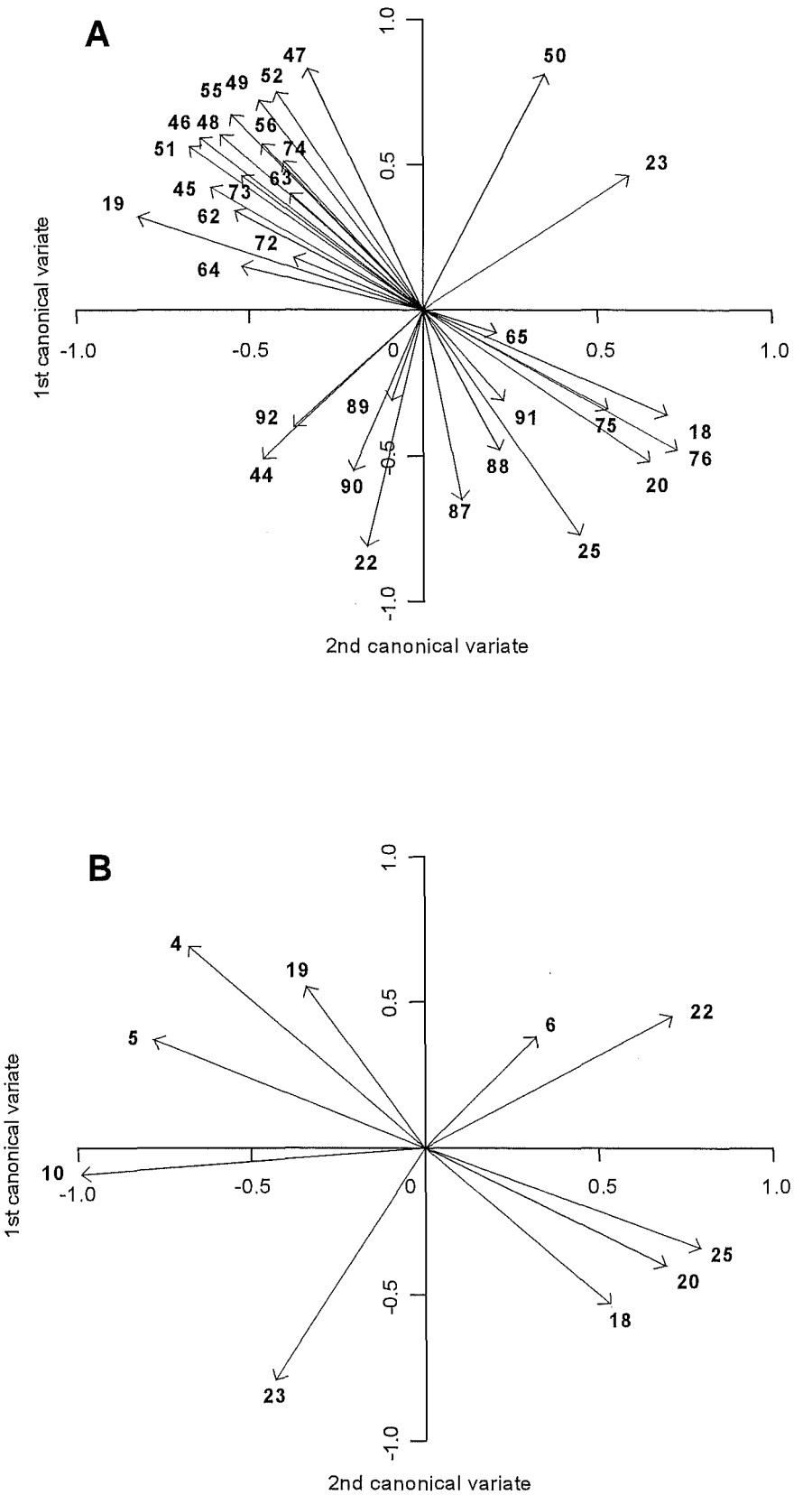


Figure 5.14. Vector plots of Pearson's product-moment correlation coefficients between the original data and the first and second canonical variates. **A**, Coefficients for an analysis of 32 continuous characters with all sympatric gnaphalioid species included (see Figure 5.13); **B**, coefficients for an analysis of 10 continuous characters with all sympatric gnaphalioid species included; characters containing outliers were excluded (see Figure 5.16).

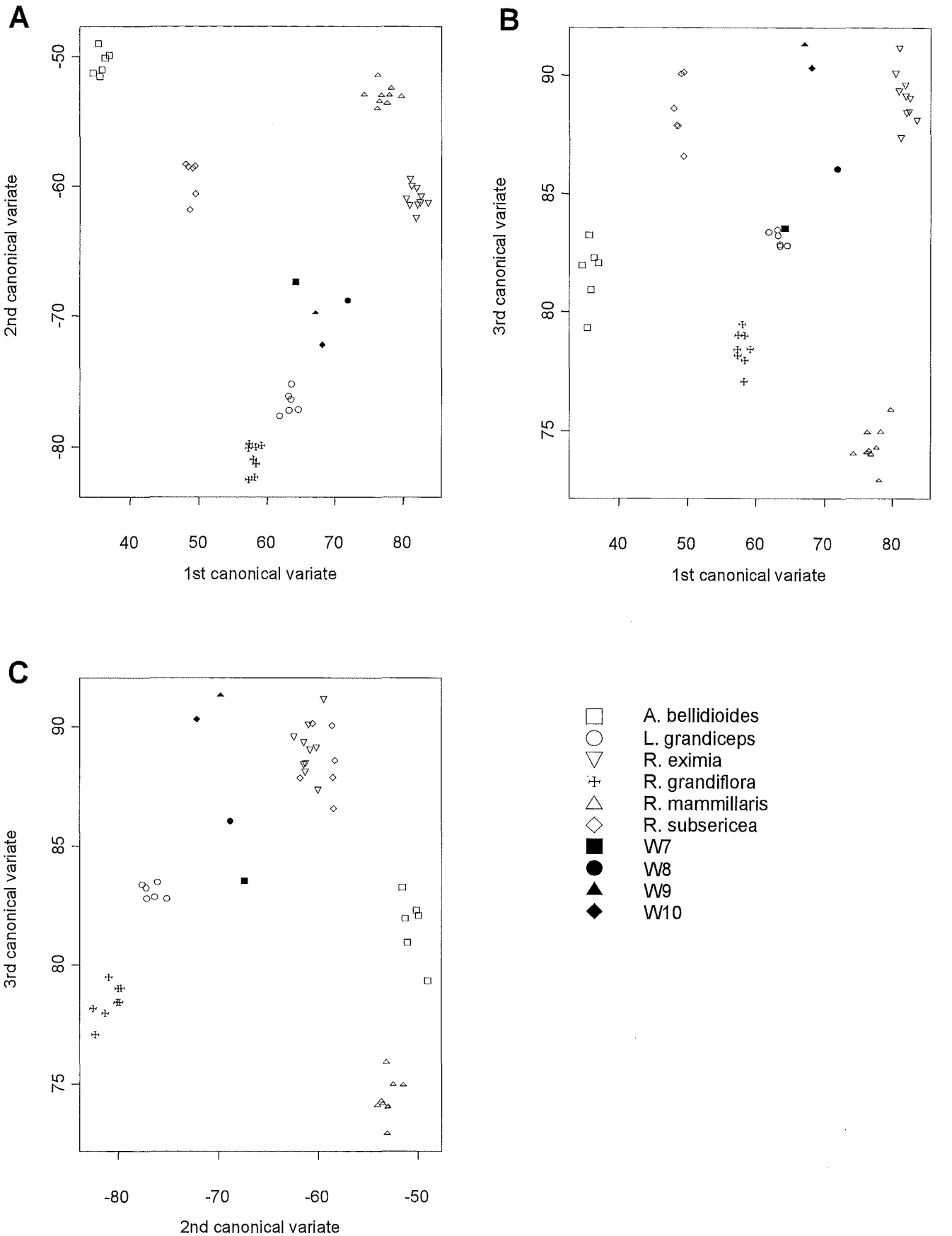


Figure 5.15. Scatter plots of the first three canonical variates for four putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia (W7, W8, W9* and *W10),* and all sympatric gnaphalioid species. 22 continuous characters were analysed. Characters containing outliers were excluded. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

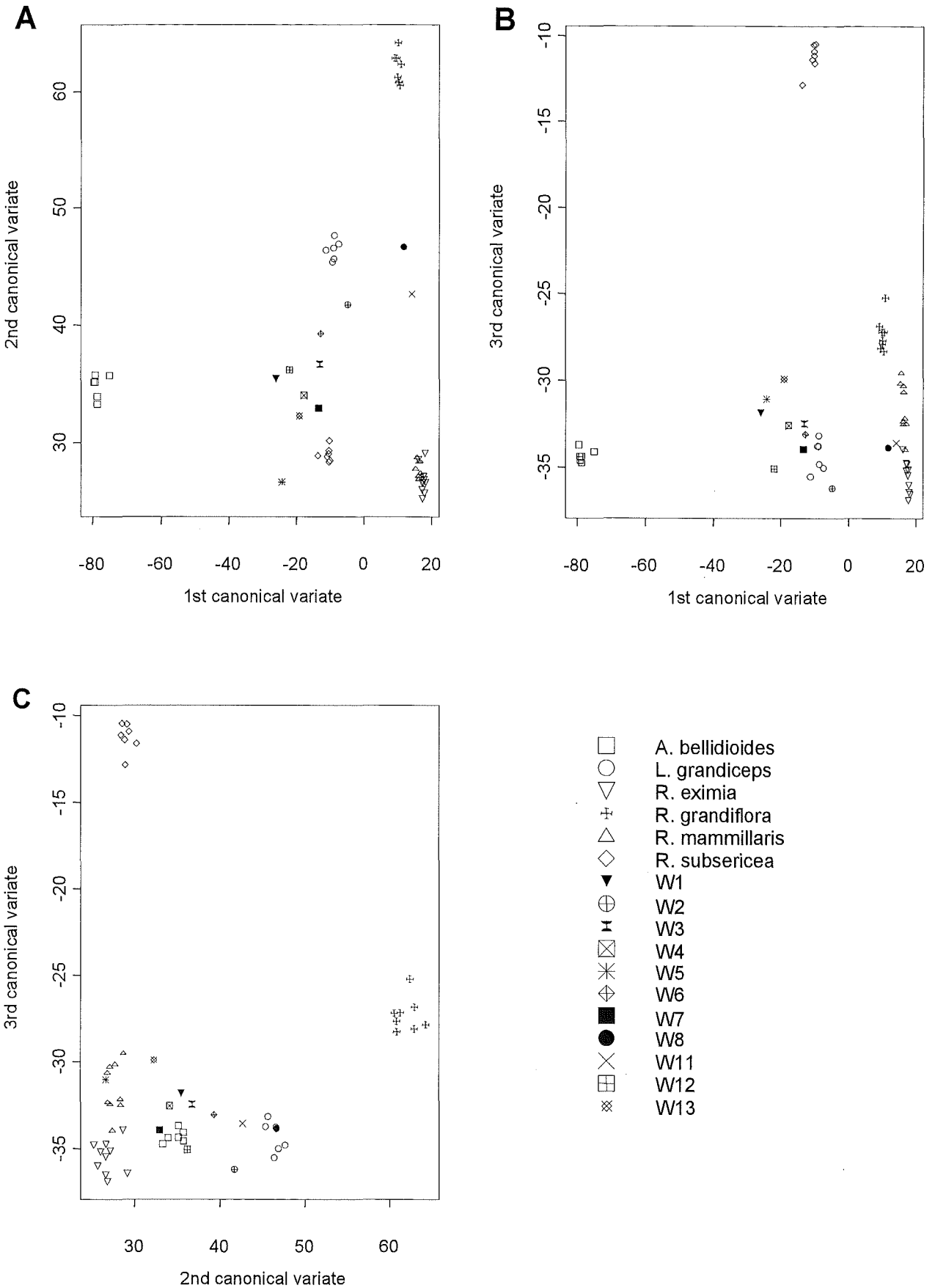


Figure 5.16. Scatter plots of the first three canonical variates for all putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* (except *W9* and *W10*), and all sympatric gnaphalioid species. Ten continuous characters were analysed. **A**, First versus second canonical variate; **B**, first versus third canonical variate; **C**, second versus third canonical variate.

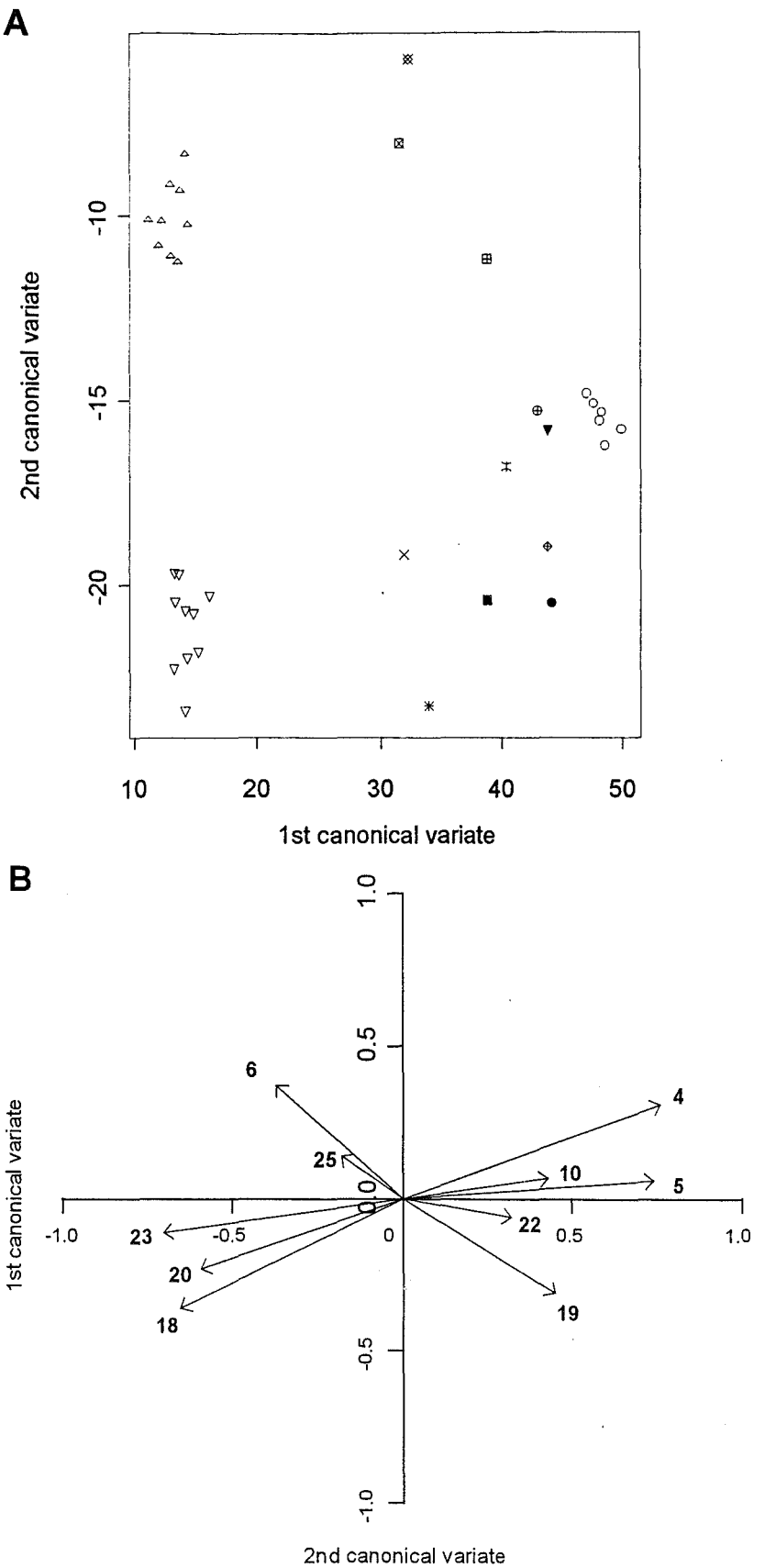


Figure 5.17. Canonical discriminant analysis of ten continuous characters for all putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia* (except *W9* and W10), the putative parental species and *R. mammillaris*. **A**, Scatter plot of the first and second canonical variates; **B**, vector plot of Pearson's product-moment correlation coefficients between the original data and the canonical variates.

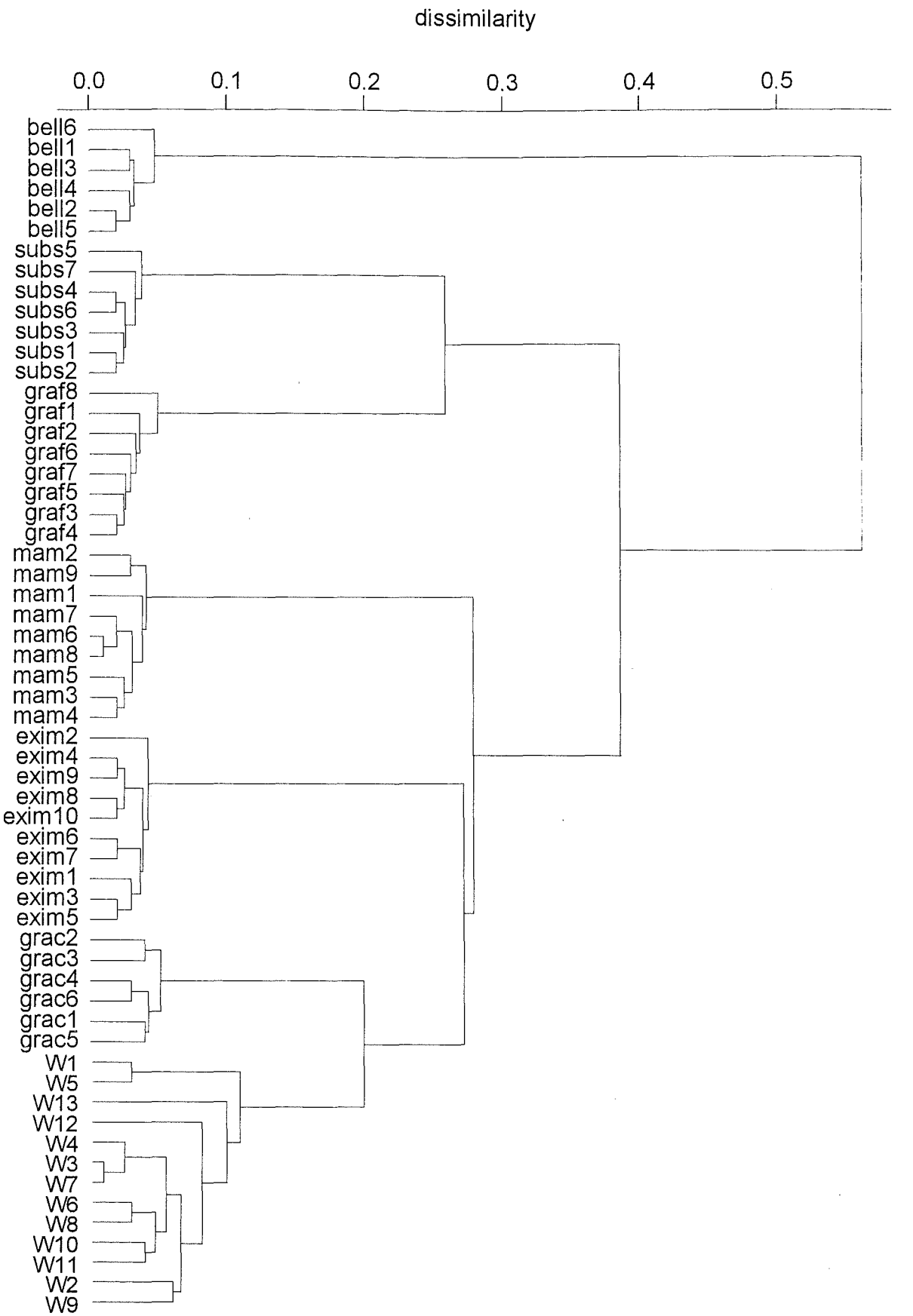


Figure 5.18. Phenogram generated by agglomerative hierarchical clustering (with group-average linkage) of dissimilarities between putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and all sympatric gnaphalioid species.

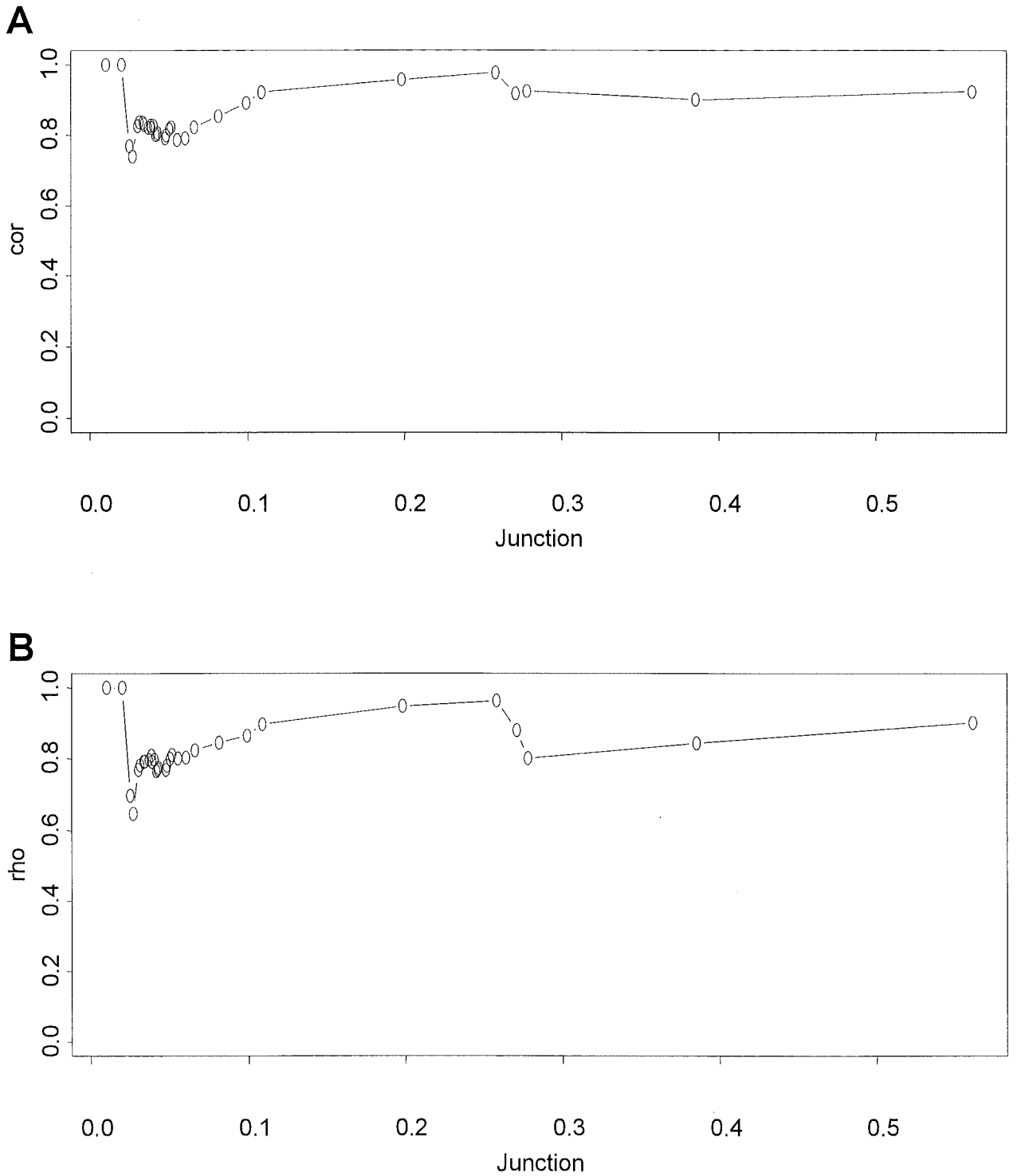


Figure 5.19. Correlation coefficients for each junction of the phenogram presented in Figure 5.18. **A,** Pearson's product-moment correlation coefficients. **B,** Spearman's rank correlation coefficients.

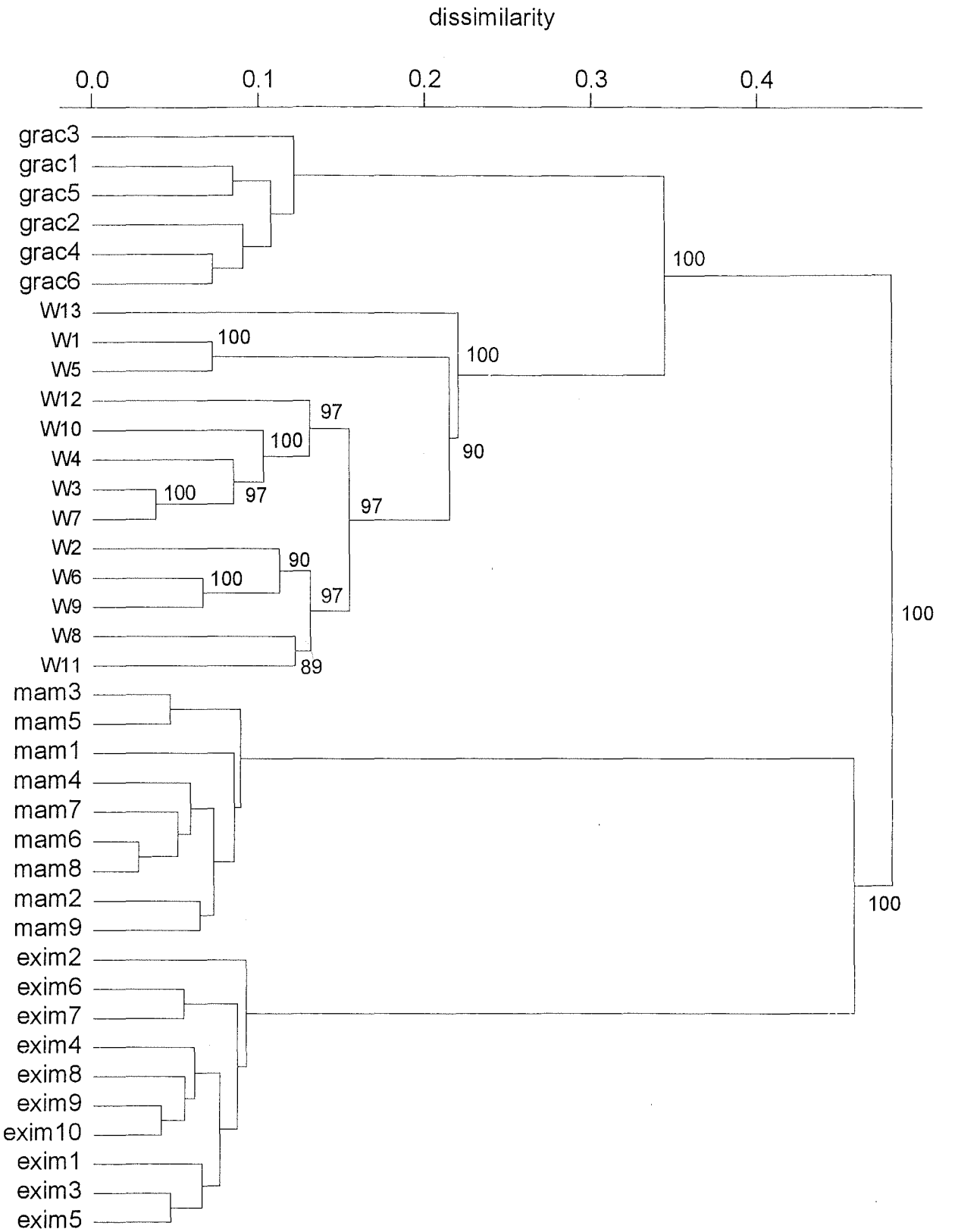


Figure 5.20. Phenogram generated by agglomerative hierarchical clustering (with group-average linkage) of dissimilarities between putatuve hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, the putative parental species and *Raoulia mammillaris*. Percentage support values from an OTU-based jackknife analysis are given for some junctions.

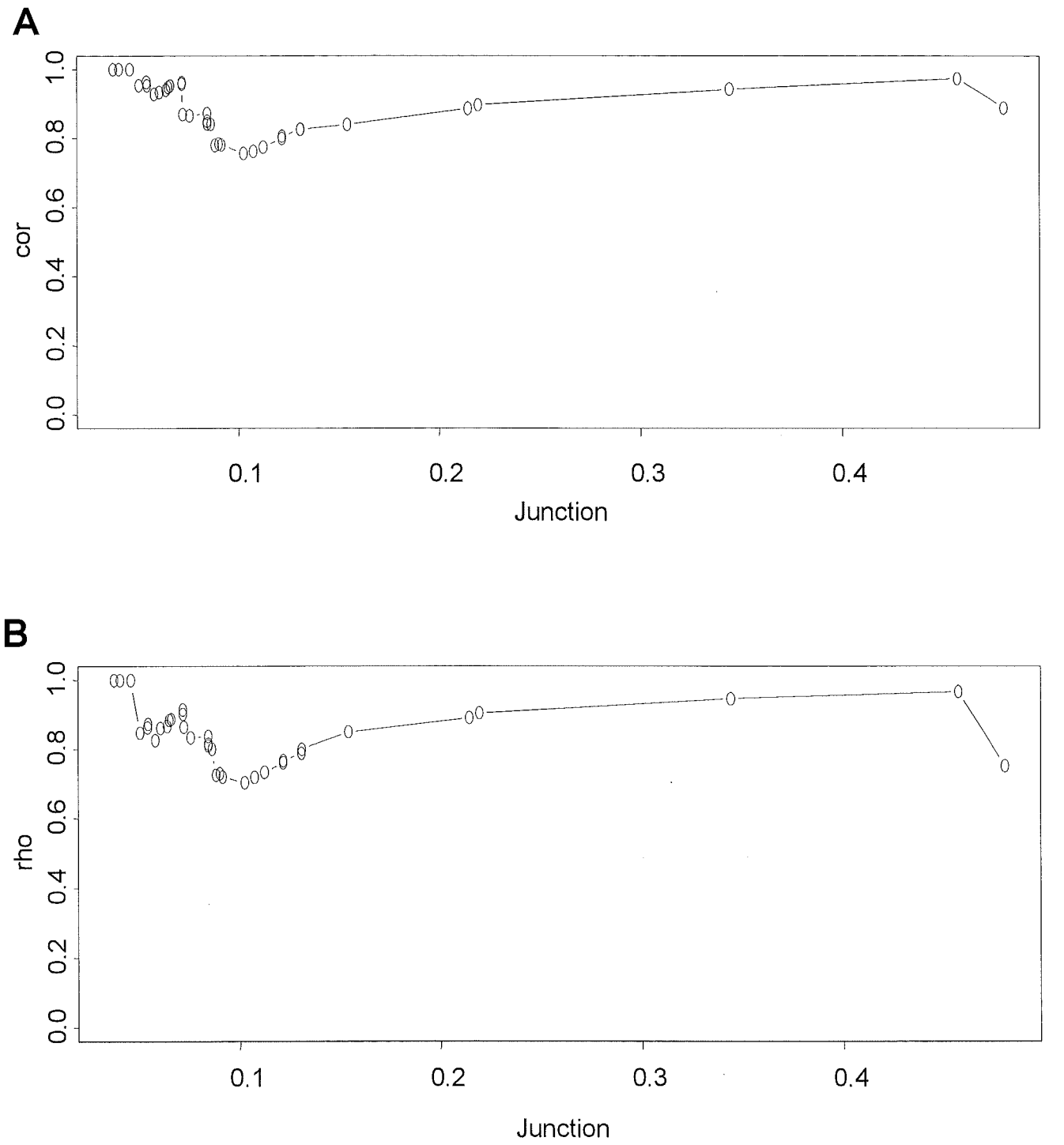


Figure 5.21. Correlation coefficients for each junction of the phenogram presented in Figure 5.20. **A,** Pearson's product-moment correlation coefficients. **B,** Spearman's rank correlation coefficients.

Weights	*W1*		*W2*		*W3*		*W4*		*W5*		*W6*		*W7*		*W8*		*W9*		*W10*		*W11*		*W12*		*W13*		*L. grandiceps*		*R. eximia*		*R. mammillaris*	
	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F	N	F
Continuous characters																																
wI=1, wE=1, wP=1	1	145	15	37	13	98	54	2	0	0	2	117	23	14	31	1	25	10	14	77	20	96	17	17	28	33	3	51	0	0	0	0
wI=0.1, wE=1, wP=1	0	0	0	0	0	0	16	82	0	0	0	0	34	6	48	10	36	1	33	39	49	2	0	0	30	17	0	0	0	0	0	0
wI=1, wE=0.1, wP=1	0	0	31	47	46	11	52	1	0	0	16	88	13	17	8	9	9	59	4	140	0	0	62	2	4	130	0	0	0	0	1	180
wI=0.1, wE=0.1, wP=1	2	205	9	21	12	27	24	1	6	82	6	50	21	2	27	5	16	4	17	18	20	9	11	6	18	14	16	30	26	38	15	70
wI=1, wE=1, wP=0.1	7	51	17	42	14	11	50	2	0	0	4	49	14	54	21	1	26	10	14	43	14	66	20	23	27	37	14	7	4	96	0	0
Mixed characters																																
wI=1, wE=1, wP=1	0	0	25	14	25	5	58	3	0	0	37	6	6	63	1	57	1	73	35	4	28	47	30	1	0	0	0	0	0	0	0	0
wI=0.1, wE=1, wP=1	0	0	0	0	0	0	23	45	0	0	53	5	42	17	11	69	7	108	57	1	21	81	23	38	9	103	0	0	0	0	0	0
wI=1, wE=0.1, wP=1	0	0	37	6	45	1	41	10	0	0	15	30	9	91	4	88	5	14	16	37	43	20	31	4	0	0	0	0	0	0	0	0
wI=0.1, wE=0.1, wP=1	0	0	21	25	22	5	23	10	5	154	23	3	18	4	15	18	15	34	24	1	20	8	24	2	15	29	0	0	0	0	21	133
wI=1, wE=1, wP=0.1	0	0	24	33	32	18	67	1	0	0	35	38	2	135	1	126	1	148	32	27	21	53	31	4	0	0	0	0	0	0	0	0
wI=1, wE=0.1, wP=0.1	0	0	38	10	51	3	63	5	0	0	12	94	5	70	1	162	3	34	5	75	43	12	25	1	0	0	0	0	0	0	0	0

Table 5.12. Summary of HYWIN analyses comparing *L. grandiceps*, *R. eximia*, *R. mammillaris* and putative *L. grandiceps* × *R. eximia* and *L. grandiceps* × *R. mammillaris*. The highest-ranking 246 hypotheses were evaluated in each analysis. Weights: wI = intermediacy; wE = equality; wP = parental distance. N = number of times ranked as a hybrid; F = rank of first time as a hybrid; 0 = never ranked.

HYBRID	PARENT	PARENT	RANK	IN	EQ	PD	NP	HS
W10	grac1	exim3	1	0.153	0.032	0.648	0.354	1.6314
W10	grac5	exim3	2	0.149	0.023	0.636	0.354	1.6274
W10	grac4	exim10	3	0.150	-0.001	0.605	0.338	1.6186
W10	grac4	exim5	4	0.177	0.002	0.597	0.336	1.6130
W6	grac5	exim2	5	0.169	-0.024	0.620	0.368	1.6129
W10	grac4	exim9	6	0.144	0.003	0.595	0.335	1.6062
W10	grac4	exim3	7	0.166	-0.024	0.613	0.338	1.6057
W6	grac1	exim8	8	0.208	-0.060	0.642	0.356	1.6031
W6	grac1	exim2	9	0.160	-0.041	0.628	0.356	1.6029
W10	grac4	exim4	10	0.131	0.002	0.591	0.337	1.6022
W10	grac1	exim5	11	0.174	0.057	0.641	0.336	1.6014
W10	grac1	exim10	12	0.160	0.055	0.640	0.338	1.6011
W10	grac4	exim8	13	0.162	0.012	0.596	0.330	1.6006
W6	grac1	exim10	14	0.200	-0.063	0.640	0.356	1.5969
W10	grac2	exim10	15	0.122	0.005	0.589	0.338	1.5963
W10	grac1	exim9	16	0.144	0.059	0.641	0.335	1.5961
W7	grac6	exim3	17	0.167	0.042	0.620	0.341	1.5954
W6	grac4	exim2	18	0.168	-0.007	0.585	0.382	1.5953
W6	grac2	exim10	19	0.183	-0.012	0.589	0.394	1.5952
W10	grac6	exim2	20	0.184	-0.016	0.592	0.319	1.5951
W10	grac2	exim9	21	0.154	0.010	0.589	0.335	1.5951
W6	grac4	exim10	22	0.187	-0.029	0.605	0.382	1.5947
W10	grac6	exim9	23	0.176	-0.025	0.602	0.319	1.5943
W6	grac1	exim9	24	0.216	-0.068	0.641	0.356	1.5941
W10	grac2	exim5	25	0.112	0.008	0.590	0.336	1.5936
W6	grac2	exim8	26	0.172	-0.009	0.585	0.394	1.5931
W6	grac5	exim3	27	0.121	-0.055	0.636	0.368	1.5927
W10	grac1	exim8	28	0.177	0.067	0.642	0.330	1.5924
W10	grac6	exim10	29	0.180	-0.030	0.604	0.319	1.5924
W10	grac1	exim4	30	0.150	0.057	0.634	0.337	1.5919
W6	grac5	exim10	31	0.156	-0.046	0.621	0.368	1.5911
W10	grac6	exim3	32	0.224	-0.052	0.620	0.319	1.5907
W10	grac6	exim5	33	0.186	-0.027	0.599	0.319	1.5906
W6	grac5	exim8	34	0.144	-0.043	0.619	0.368	1.5906
W6	grac1	exim3	35	0.134	-0.072	0.648	0.356	1.5898
W10	grac6	exim8	36	0.137	-0.017	0.593	0.319	1.5897
W6	grac2	exim9	37	0.170	-0.018	0.589	0.394	1.5886
W12	grac1	exim8	38	0.252	-0.079	0.642	0.339	1.5883
W6	grac4	exim8	39	0.171	-0.026	0.596	0.382	1.5877
W10	grac4	exim2	40	0.151	0.013	0.585	0.329	1.5873
W10	grac5	exim10	41	0.115	0.046	0.621	0.338	1.5873
W6	grac2	exim5	42	0.099	-0.013	0.590	0.394	1.5872
W6	grac1	exim5	43	0.092	-0.064	0.641	0.356	1.5870
W7	grac1	exim8	44	0.121	0.068	0.642	0.355	1.5867
W4	grac1	exim10	45	0.202	-0.073	0.640	0.346	1.5864
W6	grac4	exim3	46	0.111	-0.038	0.613	0.382	1.5863
W10	grac6	exim4	47	0.165	-0.027	0.596	0.319	1.5850
W10	grac4	exim6	48	0.106	0.008	0.582	0.332	1.5844
W10	grac4	exim1	49	0.105	0.008	0.582	0.332	1.5844
W6	grac5	exim9	50	0.162	-0.051	0.619	0.368	1.5837

Table 5.13. Results of a HYWIN analysis of 32 continuous and 61 discrete characters for *L. grandiceps*, *R. eximia*, *R. mammillaris* and putative *L. grandiceps* × *R. eximia* and *L. grandiceps* × *R. mammillaris*. The weights used were: wI = 0.1, wE = 1, wP = 1. Only the 50 highest-ranking hypotheses are listed. IN = intermediacy score; EQ = equality score; PD = parental distance score; NP = distance to the nearest parent; HS = hybrid optimality score. grac = *L. grandiceps*; exim = *R. eximia*.

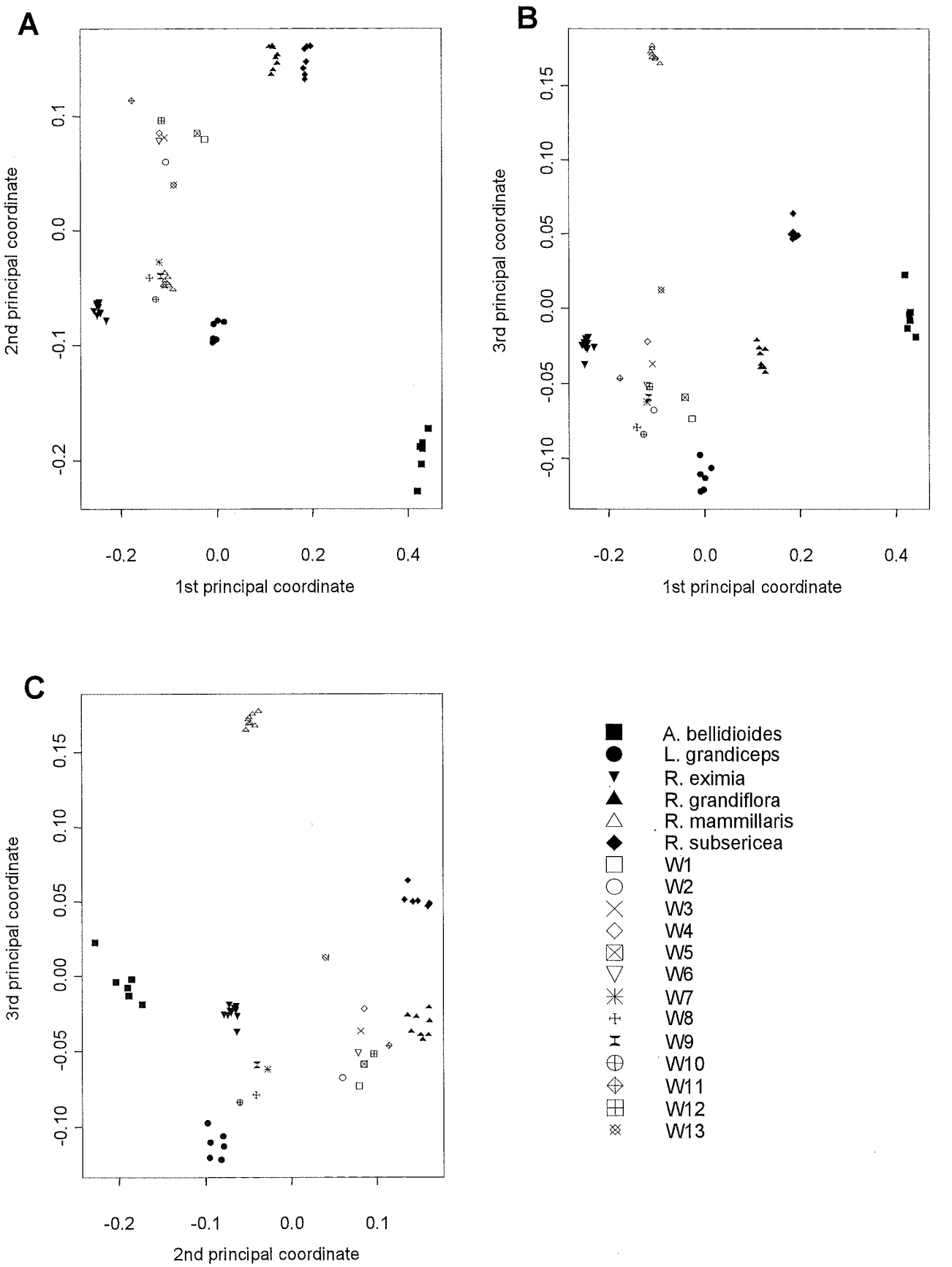


Figure 5.22. Scatter plots of the first, second and third principal coordinates for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, and all sympatric gnaphalioid species. Mixed data was analysed. **A**, First versus second principal coordinate; **B**, first versus third principal coordinate; **C**, second versus third principal coordinate.

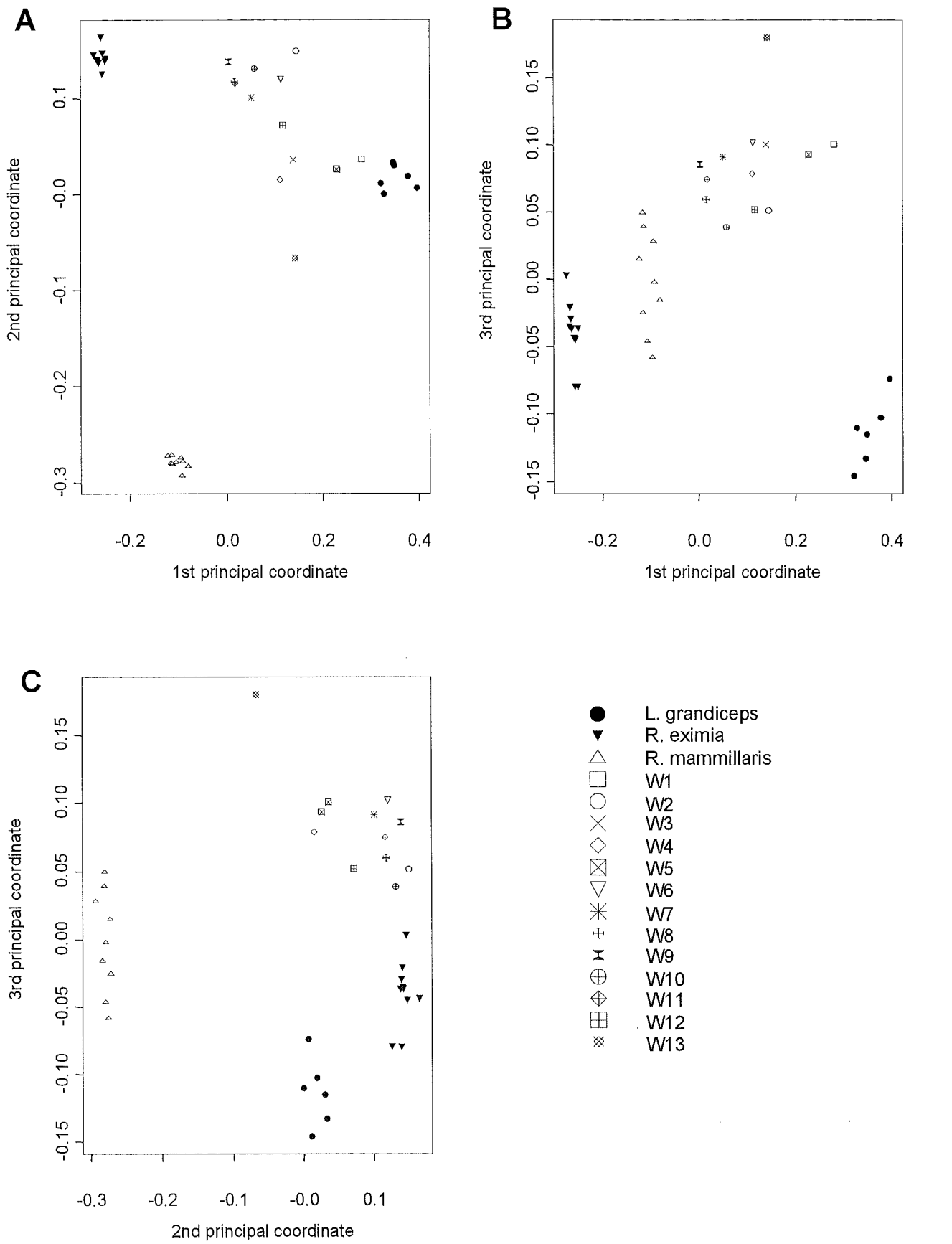


Figure 5.23. Scatter plots of the first, second and third principal coordinates for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*, the putative parental species and *Raoulia mammillaris*. Mixed data was analysed. **A**, First versus second principal coordinate; **B**, first versus third principal coordinate; **C**, second versus third principal coordinate.

Figure 5.24. Splits graphs generated by split-decomposition analysis of dissimilarities derived from mixed characters for putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia,* and all sympatric gnaphalioid species.

A, All species included (drawn to scale). Fit = 73.0 %.

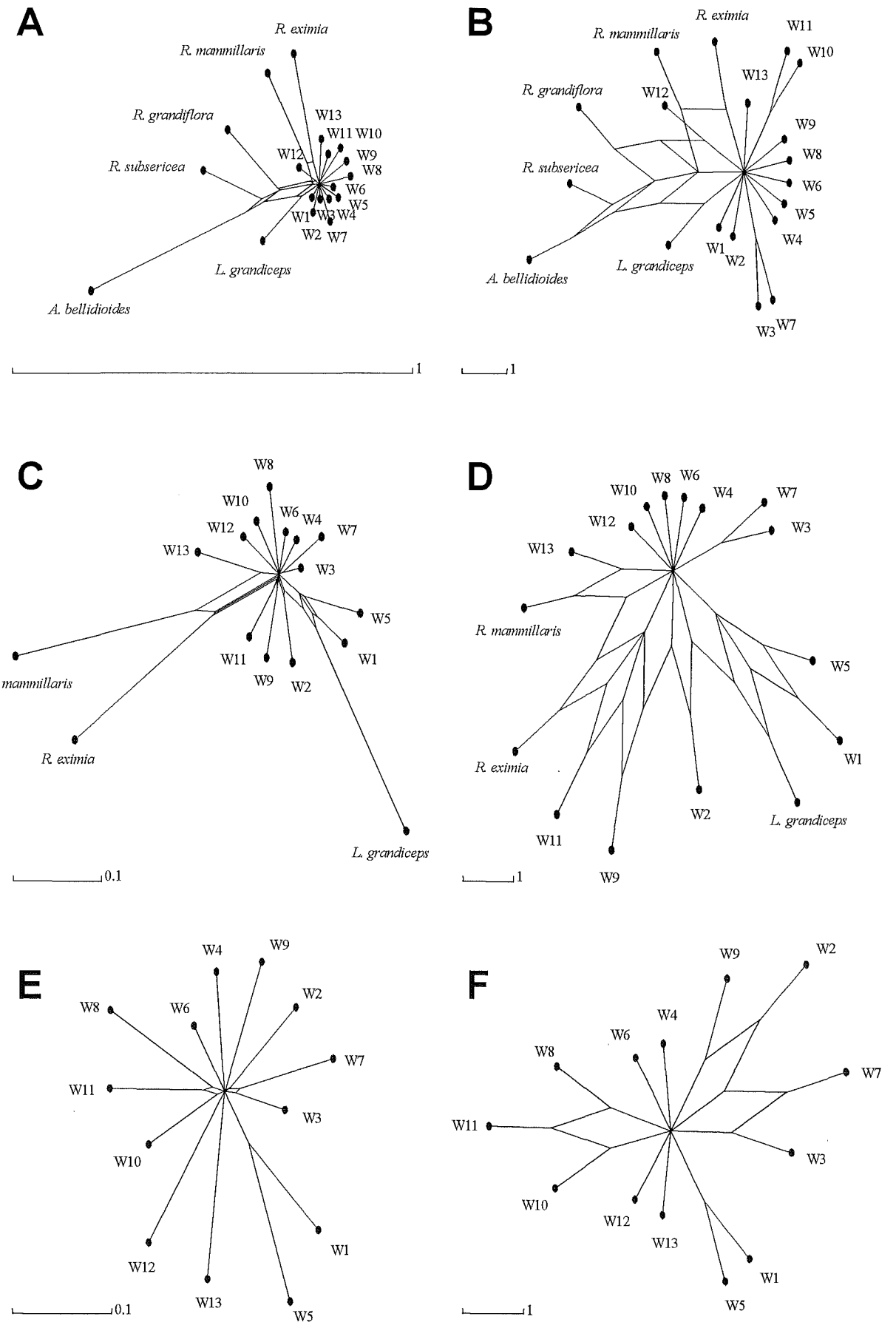
B, All species included (drawn with equal edges). Fit = 73.0 %.

C, *Anaphalioides bellidioides*, *Raoulia grandiflora* and *R. subsericea* excluded (drawn to scale). Fit = 75.4 %.

D, *A. bellidioides*, *R. grandiflora* and *R. subsericea* excluded (drawn with equal edges). Fit = 75.4 %.

E, Only the putative hybrids included (drawn to scale). Fit = 71.2 %.

F, Only the putative hybrids included (drawn with equal edges). Fit = 71.2 %.


A
R. eximia
R. mammillaris
R. grandiflora
R. subsericea
W13
W11
W10
W12
W9
W8
W6
W5
W1
W3
W4
W2
W7
L. grandiceps
A. bellidioides
1
B
R. eximia
R. mammillaris
W11
W10
W13
R. grandiflora
W12
W9
W8
R. subsericea
W6
W5
W4
W1
W2
L. grandiceps
A. bellidioides
W3
W7
1
C
W8
W10
W6
W4
W12
W7
W13
W3
W5
W11
W1
W9
W2
R. mammillaris
R. eximia
L. grandiceps
0.1
D
W8
W6
W10
W4
W7
W12
W3
W13
R. mammillaris
W5
W1
R. eximia
W2
L. grandiceps
W11
W9
1
E
W4
W9
W8
W6
W2
W7
W11
W3
W10
W1
W12
W13
W5
0.1
F
W9
W2
W4
W6
W8
W7
W11
W3
W10
W12
W13
W1
W5
1

5.3.5 Experimental crosses

In two *L. grandiceps* × *R. eximia* crosses (employing capitula from different *L. grandiceps* plants), the proportion of germinated *R. eximia* pollen grains was 8.6 % and 24.1 %. All germinated grains were attached to the stigma. In a reciprocal cross (*R. eximia* × *L. grandiceps*), only four pollen grains out of 310 had germinated. Filled cypselas were obtained from one *L. grandiceps* × *R. eximia* cross, but no cypselas enlarged in one *R. eximia* × *L. grandiceps* cross and *R. eximia* × *W9* (Table 5.14).

5.4 Records of other wild hybrids

CHR 108586

This sterile specimen was collected from Mt Torlesse, Torlesse Range by Walter Brockie in January 1941. The leaves were oblong-obovate with a rounded apex and a short mucro. Only a trinervate leaf was examined. The clothing trichome terminal cells projected well beyond the leaf apex, were loosely interwoven and appressed and somewhat undulate on the adaxial surface. The terminal cell width was variable (9–26 μm wide) and only two basal cells were observed. The glandular trichomes and leaf anatomy was identical to the Mt Hutt putative hybrids and no sclerenchyma fibre caps were present on the vascular bundles. Thus, based on vegetative morphology this plant appears to be of the same origin as the Mt Hutt putative hybrids.

CHR 285223

This specimen was collected from the Ohau Ski Basin by Margaret Simpson on 2 December 1972. The plant was recorded as growing on rock at about 1700 m. The sheet contains a single sterile branch with long adventitious roots. The shoots formed a hard cushion but with

Maternal parent × Paternal parent	Date	No. of capitula and florets pollinated		Style retraction	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		Capitula	florets					
R. eximia A/2 × *W9*	16/1/98	5	all ♀	0	0	0	c.20	0
R. eximia A/2 × *L. grandiceps* A/5	16/1/98	8	all ♀	0	0	0	30	0
L. grandiceps A/5 × *R. eximia* A/5	16/1/98	8	all ♀	NA	12	0	90	12

Table 5.14. Results of artificial crosses between plants of *Leucogenes grandiceps*, *Raoulia eximia* and a putative hybrid between the two species.

an uneven surface (unlike *R. eximia*). The trinervate leaves were oblong-obovate with a rounded apex and a rudimentary mucro. The indumentum and leaf anatomy was similar to that of the Mt Hutt putative hybrids. The clothing trichomes had 2–3 basal cells and the terminal cell was variable in width (12–22 μm). Sclerenchyma fibres were not observed on the vascular bundles.

Tripps Peak

This plant was discovered on Tripps Peak, Four Peaks Range by Brian Molloy on 24 April 1979. The uni- to trinervate leaves were oblong-obovate with an obtuse apex and a short mucro. The leaf indumentum was very similar to that of the Mt Hutt putative hybrids. The clothing trichomes had 2–3 basal cells and terminal cells of variable width (6–23 μm). The leaf anatomy was more similar to that of *R. eximia*; the mesophyll was less differentiated into three layers and large sclerenchyma fibre caps were present on the adaxial side of the vascular bundles. The vegetative morphology suggests this plant is a *L. grandiceps* × *R. eximia* hybrid, but in leaf anatomy it is more similar to *R. eximia*.

Coal Hill

This plant was collected from Coal Hill, Tara Haoa Range by Brian Molloy on 1 May 1979. The leaves were bi- or trinervate, oblong-obovate with a rounded apex. The indumentum and leaf anatomy was similar to that of the Mt Hutt putative hybrids. The clothing trichomes had 2–3 basal cells and the terminal cell width was variable (8–22 μm). Up to three sclerenchyma fibres were present on the adaxial side of the midrib.

CHR 404353

This specimen was collected from the Ohau Ski Basin by Margaret Simpson on 9 February 1980. The plant was recorded as growing on a rocky bluff at 2000 m. The sheet contains a single sterile shoot. Its vegetative morphology was similar to CHR 285223 (collected from the same locality), but differed in the following characters: only trinervate leaves with a short mucro were observed; the clothing trichomes had 2 basal cells; and up to two sclerenchyma fibres were observed on the adaxial side of the midrib. Thus the two specimens appear to be of the same parentage.

Mt Potts

This plant was collected from Mt Potts, Canterbury by Aaron Wilton and cultivated at the University of Canterbury. The vegetative morphology and leaf anatomy of the cultivated

clone was very similar to the putative *L. grandiceps* × *R. eximia* hybrids from Mt Hutt. The leaves were oblong-obovate, 5–5.6 mm long × 2.9–3.7 mm wide with a rudimentary mucro. Only trinervate leaves were observed. The terminal cell of most clothing trichomes was appressed and interwoven and only slightly projected beyond the leaf apex. The clothing trichomes had two (rarely three) basal cells, which were 50–95 μm long and 10–15 μm wide with similar wall thickness to *L. grandiceps*. The type A glandular trichomes were biseriate, 129–184 μm long, with oblong terminal cells 27–31 μm long. The mesophyll consisted of three distinct layers and the central chlorenchyma cells were 30–55 μm wide. Sclerenchyma fibres were not observed on the vascular bundles.

Big Mt Peel

I collected this non-flowering specimen from Big Mt Peel, Tara Haoa Range, Canterbury on 7 April 1998. It was growing in a shingly gut between two bluffs. Both putative parental species were common at the site. The related *L. tarahaoa* was separated altitudinally. The vegetative morphology of the field-grown specimen was very similar to the putative *L. grandiceps* × *R. eximia* hybrids from Mt Hutt. The leaves were oblong, 3.5–4.2 cm long × 2.4–2.7 mm wide with a rounded apex and a mucro 4 μm long. Only trinervate leaves were observed. The leaf indumentum was similar to that of *R. eximia*, in that the terminal cell of most clothing trichomes was only loosely appressed and interwoven on both lamina surfaces and projected prominently beyond the leaf apex and margins. However, the terminal cell was narrower (8–14 μm wide). The clothing trichomes had two or three basal cells, which were 50–67 μm long and 10–16 μm wide, and slightly thicker walled than in *L. grandiceps*. The leaf glandular trichomes were biseriate, 130–205 μm long, with oblong terminal cells 30–35 μm long. Leaf anatomy was identical to most of the Mt Hutt putative hybrids, but one to four sclerenchyma fibres were present on the adaxial side of the midrib and major lateral veins. The mesophyll consisted of three distinct layers and the central chlorenchyma cells were 30–40 μm wide.

5.5 Discussion

Evidence for hybridity

Morphological data and field evidence supported the hypothesis that the putative hybrids were crosses between *Leucogenes grandiceps* and *Raoulia eximia*, but were inconclusive with respect to confidently characterising the hybrids as F_1, later-generation or backcross progeny. The putative hybrids lacked novel characters and extreme characters were rare. Continuous characters in the putative hybrids were predominantly intermediate between the putative

parental species and discrete characters were principally present in parental states. Meiotic pairing in microsporocytes and pollen stainability in *W9* indicated fertility was only slightly reduced and so were less supportive of the hybridity hypothesis. Floral characters provided particularly strong evidence for hybridity for four putative hybrids. Three sympatric species (*A. bellidioides*, *R. grandiflora* and *R. subsericea*) were discounted as possible parents on morphological evidence, but the morphological and anatomical similarity of *L. grandiceps*, *R. eximia* and *R. mammillaris* restricted the number of characters useful for detecting hybridity and determining the most likely parental species.

The floral morphology of *W7*, *W8*, *W9* and *W10* strongly implicated *L. grandiceps* and *R. eximia* as the parental species. Characters unique to *L. grandiceps* expressed in the putative hybrids were multicapitulate inflorescences and yellow corolla-lobe apices. Floral characters unique to *R. eximia* expressed among the putative hybrids included crimson corolla lobes, and an acute apex and pale brown lamina on the inner involucral bracts. Two unique floral characters recorded for *R. mammillaris* were the presence of reduced style arms and a reduced ovary in some hermaphrodite florets (the two characters were not always correlated), but neither were expressed in the putative hybrids. This is consistent with the findings of Wilton (1997), who reported that seed set in *R. mammillaris* plants from the Craigieburn Range, central Canterbury was extremely low in hermaphrodite florets (2 %) compared to female florets (87 %). Wilton (1997 p. 229) suggested that selection for functionally unisexual florets might be occurring in *R. mammillaris*. Based on morphology, there was no indication of functional differences in the hermaphrodite florets of *L. grandiceps*, *R. eximia* or the putative hybrids *W7*, *W8*, *W9* and *W10*.

Anaphalioides bellidioides, *R. grandiflora* and *R. subsericea* possessed numerous unique characters (e.g., leaf shape and leaf trichome characters) not expressed in the putative hybrids. A number of characters were recorded only among *A. bellidioides*, *R. grandiflora*, *R. mammillaris* and *R. subsericea* and were not expressed in the putative hybrids, including white or pale green corolla lobes, a white lamina on the inner involucral bracts, and reticulate wall thickening in the pappus-hair apical cells. The intermediacy of *R. mammillaris* between *L. grandiceps* and *R. eximia* in some characters, and the sharing of some distinctive characters between *R. eximia* and *R. mammillaris*, hindered determination of the putative hybrids. The possession of both type A and B clothing trichomes, and of appressed and non-appressed clothing trichomes, on the leaves of most of the putative hybrids suggested an affinity with *R. mammillaris*. However, the distribution of sclerenchyma fibre caps on the vascular bundles,

the number of clothing-trichome basal cells and number of leaf traces discriminated the putative hybrids from *R. mammillaris*. The presence of sclerenchyma fibres on the adaxial side of vascular bundles provided evidence for an affinity between *R. eximia*, *W12* and putative hybrids from Coal Hill, Mt Peel, the Ohau Ski Basin and Tripps Peak. Idioblastic sclereids in the petiole, a feature of *R. eximia* and *R. mammillaris*, were not observed in any putative hybrid. *W1* and *W5* possessed a greater frequency of *L. grandiceps* vegetative characters than the other putative hybrids, principally due to the absence of type B clothing trichomes on the leaves. *W13* (a putative hybrid between *L. grandiceps* and *R. mammillaris*) differed from the other putative hybrids in the narrower clothing-trichome terminal cells and in possessing clothing trichomes with one or two basal cells, characters shared with *R. mammillaris*, but was otherwise morphologically similar to these plants. The number of primary leaf traces was consistently one or three in the sympatric species, but was variable in all putative hybrids except *W9*. This was not a function of leaf size and appeared not to be related to developmental stage (e.g., representing a transition between true leaves and involucral bracts), although additional investigation is required to confirm the latter. Consequently, it is interpreted as an intermediate state and as evidence for hybridity between species with uninervate and trinervate leaves. This character thus supported the hypotheses of hybridity between the trinervate *L. grandiceps* and uninervate *R. eximia* and *R. mammillaris* for all putative hybrids except *W9*.

Analyses of the morphological data suggested conflicting hypotheses regarding the identity of the putative hybrids. MDS and HYWIN analysis of mixed characters with a low intermediacy weighting strongly supported the *L. grandiceps* × *R. eximia* parentage hypothesis. CDA was generally supportive of this parentage but placement of the putative hybrids varied depending on the composition of the data set. CDA, hierarchical clustering, MDS and split decomposition strongly suggested *A. bellidioides*, *R. grandiflora* and *R. subsericea* were unlikely to be parents. Exclusion of these species strengthened the suggestion that *W1* to *W12* were hybrids between *L. grandiceps* and *R. eximia*. Hierarchical clustering always linked the putative hybrids to *L. grandiceps*, although the raw dissimilarities showed that *W7*, *W8*, *W9* and *W10* were more similar to *R. eximia* than *L. grandiceps*. HYWIN analyses of the complete data set and of continuous characters suggested a wide variety of parentage hypotheses and species individuals were often ranked as hybrids, indicating the results should be interpreted with caution. Greatest support by HYWIN for the *L. grandiceps* × *R. eximia* parentage hypothesis occurred in analyses of mixed data with a low equality or intermediacy weighting and with the least likely parental species excluded. In splits graphs the putative

hybrids did not form a single group defined by an internal edge and were closely associated with *L. grandiceps* and *R. eximia*, but *R. mammillaris* could not be excluded as a possible parent. MDS provided the stongest support for the hypothesis that *W13* was a *L. grandiceps* × *R. mammillaris* hybrid. CDA and split decomposition provided weak support for this hypothesis. Hierarchical clustering always grouped *W13* with the other putative hybrids, although with most linkage methods it was the most dissimilar individual in the cluster.

Characterisation of the putative hybrids

Confident characterisation of the putative hybrids (as possible F_1, later-generation or backcross progeny) was not possible, owing to the degree of variation among the specimens, the absence of consistent groupings in data analyses and the unavailability of floral characters for all but four of the putative hybrids. It is likely molecular genetic techniques, such as isozyme analysis and microsatellite markers, would provide improved resolution of relationships among the putative hybrids and putative parental species (see Chapter 7). However, morphology provided some evidence for the presence of backcross or later-generation hybrids among the putative hybrids studied.

The absence of type B clothing trichomes set *W1* and *W5* apart from the other putative hybrids and implied *W1* and *W5* were closer to *L. grandiceps* than were the other putative hybrids. *W1* and *W5* were shown to be most similar to *L. grandiceps* among the putative hybrids by all methods except CDA. The CDA results were not unexpected, as the characters suggesting a close similarity with *L. grandiceps* were discrete and therefore excluded from the canonical discriminant analyses. Raw dissimilarities indicated *W1* and *W5* were marginally more similar to *R. mammillaris* than to *R. eximia*, but the raw values were not as low as those between *W13* and *R. mammillaris* and no analytic method strongly implicated *R. mammillaris* as a possible parent of *W1* and *W5*. Both *W1* and *W5* were immature plants and one interpretation is they were backcrosses with *L. grandiceps* and *R. eximia* was the most likely other parental species.

In vegetative characters *W7*, *W8*, *W9* and *W10* were extremely similar and differed principally in leaf shape and leaf apex shape, but floral characters were more variable and suggested a closer affinity with *R. eximia* than with *L. grandiceps*. MDS suggested *W7*, *W8*, *W9* and *W10* had a close affinity but they were never grouped by the other analytic methods. *W8* differed from *W7*, *W9* and *W10* in three floral characters (corolla lobe apex colour, involucral bract apex shape and receptacle height) that suggested it was more similar to *R. eximia* than for the

other three putative hybrids. This affinity was represented in some canonical variates but was not consistent. *W10* was more similar to *L. grandiceps* than *W7*, *W8* and *W9* in a number of floral characters (e.g., receptacle dimensions, floret numbers, pappus hair length and female-floret corolla tube length), but a closer affinity with *L. grandiceps* was not suggested by any analytic method used. However, vegetative characters were less suggestive of a closer affinity with *R. eximia* for these four putative hybrids.

Cluster analysis consistently grouped *W3*, *W4* and *W7*. Character counts, character indices and MDS represented *W3* and *W4* as being similar. Splits graphs linked *W3* and *W7*. However, CDA did not suggest these plants were notably similar. Relationships among the other putative hybrids were not clearly resolved. Character counts and character indices suggested *W2* was very similar to *W3* and *W4*, but the other analytical methods provided little support for such a relationship. Marked differences in the expression of discrete and continuous characters partly explained the variable relationships suggested by the different analytic methods for *W5*, *W6*, *W8* and *W11*.

On some canonical variates, some putative hybrids were non-intermediate between the putative parental species, but this cannot be due solely to environment-induced variation, as even vegetative characters recorded from cultivated clones proved variable. In addition, the greater variation among the putative hybrids than within each species indicates a genetic contribution to the variability. Extreme characters were rare and confined to *W6*, *W7* and *W8*, and no novel character was identified among the putative hybrids. Extreme and novel characters tend to be more common in later-generation hybrids (Rieseberg and Ellstrand, 1993). The most variable discrete characters were leaf shape, leaf apex shape and the morphology of the leaves subtending the capitulum, each of which might be expected to exhibit a certain degree of variation in F_1 hybrids. The leaf indumentum of the putative hybrids was either intermediate between the putative parents or more similar to *L. grandiceps*. In no putative hybrid was the indumentum most similar to that of *R. eximia*.

Thus, overall, the morphological and anatomical evidence supported the hybridity hypothesis and *L. grandiceps* was implicated as a putative parent on vegetative morphology. Floral characters provided strong evidence that four putative hybrids were crosses between *L. grandiceps* and *R. eximia*, and vegetative similarities suggested the remaining putative hybrids were of the same origin or crosses between *L. grandiceps* and *R. eximia*. However, confident characterisation of the putative hybrids as possible F_1, later-generation or backcross

progeny was not possible. Floral characters suggested *W7*, *W8*, *W9* and *W10* might be backcrosses with *R. eximia* but vegetative characters were less supportive of this hypothesis. *W1* and *W5* might represent backcrosses to *L. grandiceps*. The inconclusive results in some analyses partly reflect the unavailability of floral characters for nine putative hybrids and the lack of distinct groups among the putative hybrids (unlike case study 1). Discrimination of *W13* (a putative hybrid between *L. grandiceps* and *R. mammillaris*) from the other putative hybrids solely on vegetative characters was difficult, but the clothing-trichome terminal cell width and number of clothing-trichome basal cells suggested *W13* had a closer affinity to *R. mammillaris* than the other putative hybrids.

Putative hybrids from other localities

Vegetative morphology suggested seven non-flowering specimens collected from six other localities in central and southern Canterbury were of the same parentage as the putative hybrids between *L. grandiceps* and *R. eximia* from Mt Hutt. All of the localities lie within the geographical range of both putative parents and of these localities *R. mammillaris* occurs only on Mt Torlesse (J. M. Ward, pers. comm.). No information on the frequency of the putative hybrids at the other localities was provided by other collectors, but on Big Mt Peel I located only a single individual. The morphological similarity of most putative hybrids from these localities, their overall intermediacy between the putative parents and their apparent rarity in the field might suggest the majority were F_1 hybrids. The putative hybrid from Tripps Peak was the most divergent of those studied. Certain characters were closer to *R. eximia* (leaf anatomy) or to *L. grandiceps* (leaf apex shape) than for the other putative hybrids. Greater mixing of parental characters would be expected in later-generation hybrids due to segregation, therefore the Tripps Peak plant might be a F_2 or later-generation hybrid. The parental species are not uncommonly sympatric in Canterbury, so it is possible putative hybrids also occur in other localities. However, it is apparent that, despite considerable botanical exploration over the preceding 150 years, putative hybrids between *L. grandiceps* and *R. eximia* have been rarely encountered in the field and the present study appears to be the first recorded collection of flowering specimens of these plants.

Factors influencing hybridisation in the field

At the study site *L. grandiceps*, *R. eximia* and the putative hybrids had coincident flowering periods (January–February) and the heterogeneity of the site allowed both species and putative hybrids to grow in close proximity, thereby enhancing opportunities for interbreeding. Only four putative hybrids at the site were found in flower and these plants did

not flower every year. The putative hybrid *W9* had high pollen stainability, but the capacity of the pollen grains to achieve *in vivo* fertilisation is unknown. As mentioned previously (p. 190), competition between conspecific and heterospecific pollen could restrict generation of F_1 hybrids, and pollen from either hypothesised parental species might outcompete hybrids' pollen, thereby limiting the production of backcross and later-generation hybrids. A variety of generalist insect visitors have been observed on *L. grandiceps* capitula: solitary bees (Apoidae), Coleoptera, Lepidoptera, Syrphidae, Tachinidae and other unidentified Diptera (Primack, 1983; Wilton, 1997). No studies of insect visitors to *R. eximia* capitula have been published.

Hybrid fitness is likely to be an important factor for these hybrids, particularly their tolerance of moisture on the foliage (which encourages fungal growth and might disrupt photosynthesis and transpiration) and water penetration into the centre of the plant (which might promote stem and root rot). The leaves of both putative parents have a dense indumentum on the adaxial lamina surface; in *L. grandiceps* it is relatively water-repellent, but *R. eximia* is principally reliant on its cushion growth form and tightly packed shoots to discourage moisture from accumulating on the leaves. In most of the putative hybrids the terminal cell of some clothing trichomes was not appressed to the leaf surface and in many plants the shoots were less tightly packed than in *R. eximia*. Thus water accumulation on the foliage could be greater in hybrids than either species and thus limit the number of hybrids or particular phenotypes able to become established in the field. None of the putative hybrids possessed indumentum similar to *R. eximia*; such plants might be poorly adapted and die at an early stage in the field. The putative hybrids are slow growing and difficult to maintain in cultivation at low altitudes, seemingly due to sensitivity to heat, humidity and soil-moisture levels. Therefore relatively specific environmental conditions might be required for a hybrid to establish in the field, such as a free-draining substrate that does not become overly dry in summer, avoidance of competition with more vigorous plants, an open situation and avoidance of shading from larger plants, and exposure to air currents to discourage moisture from accumulating on the foliage. The substrate instability at the study site would help to reduce competition with more vigorous and shallow-rooting plants, but would also hinder establishment of hybrid seedlings. Such conditions might similarly constrain establishment of *R. eximia* seedlings in the field, which are rarely encountered at any site (J. M. Ward, pers. comm.). It appears flowering specimens of putative hybrids between *L. grandiceps* and *R. eximia* have not been collected previously and Allan (1961 p. 713) noted that flowering specimens of putative hybrids between any species of *Leucogenes* and *Raoulia* subg.

Psychrophyton were rare, which might indicate few such hybrids survive to maturity in the field.

In conclusion, field evidence and morphology supported the hybridity hypothesis, and four putative hybrids for which floral characters were available were strongly implicated to be hybrids between *L. grandiceps* and *R. eximia*. A putative hybrid between *L. grandiceps* and *R. mammillaris* (*W13*) was discriminated from the other putative hybrids on leaf-trace and leaf-trichome characters, but was otherwise very similar in morphology. Characterisation of the putative hybrids from morphology was difficult, but two putative hybrids (*W1* and *W5*) had a greater resemblance to *L. grandiceps* and could represent backcross hybrids. Pollen stainability and meiotic pairing in microsporocytes indicated one of the putative hybrids (*W9*) was of relatively high fertility and suggested *L. grandiceps* and *R. eximia* have a high genetic affinity. This also indicates potential for later-generation hybrids and backcrosses to arise. Various factors might be responsible for the scarcity of both mature and immature hybrids at the site, including a low frequency of hybridisation owing to pollinator differences or pollen competition, substrate instability, availability of suitable locations, and low hybrid fitness.

SECTION THREE

Experimental Hybridisation of New Zealand Gnaphalieae

Chapter 6. Experimental hybridisation of New Zealand Gnaphalieae

6.1 Introduction

Numerous studies of the New Zealand flora have utilised experimental crosses (Connor, 1985; Hair, 1966). Extensive artificial crosses have aided resolution of relationships among New Zealand *Epilobium* (see Raven and Raven, 1976), Gramineae (see Connor, 1985) and alpine species of *Ranunculus* (Fisher, 1965). Moore (1980) raised artificial intergeneric hybrids between species of *Astelia* and *Collospermum*. Natural or experimental hybrids between native and exotic species are recorded in *Acaena* L. (Macmillan, 1988), *Astelia* & *Collospermum* (Moore, 1980), *Cortaderia* Stapf (Connor, 1983), *Elymus* (Löve and Connor, 1982), *Epilobium* L. (Raven and Raven, 1976), between *Disphyma australe* and two adventive *Carpobrotus* species (Chinnock, 1972), in *Luzula* (Nordenskiöld, 1971) and between Australian and New Zealand members of the *Senecio glaucophyllus* complex (Ornduff, 1962).

Artificial crosses have been often utilised to aid resolution of taxonomic and phylogenetic relationships in the Compositae. For example, the experimental cross-compatibility of *Helianthus porteri* with four *Helianthus* species and the synthesis of only a single, sterile hybrid with *Viguiera adenophylla* (Heiser, 1963) supported the subsequent transfer of *H. porteri* from *Viguiera* to *Helianthus* (Yates and Heiser, 1979). Cross-compatibility has supported the merging of some genera (e.g., Olorode and Torres, 1970; Powell, 1972), but numerous cross-compatible genera are accepted in the Compositae (see Chapter 2) and some authors have segregated new genera despite their cross-compatibility with other genera (e.g., Baldwin, 1999). Babcock (1947) found that differences in cross-compatibility, hybrid vigour and fertility generally agreed with his sectional classification of *Crepis*. Experimental hybrids have been utilised to study the genetic basis of phenotypic characters, such as receptacular scales in *Crepis* (Babcock and Cave, 1938; Babcock, 1947) and self-compatibility in *Stephanomeria* Nutt. (Brauner and Gottlieb, 1987). Numerous studies have investigated chromosome pairing in hybrids (e.g., Clausen *et al.*, 1945; Mitsuoka and Ehrendorfer, 1972; Carr and Kyhos, 1986) as a measure of homology between the parental species. Artificial crosses have also demonstrated that in some groups barriers to hybridisation are weak or absent and that pre-zygotic factors (e.g., ecological, geographical or flowering separation) are most important in reproductive isolation (e.g., Ganders and Nagata, 1984; Carr *et al.*, 1996;

Brochmann *et al.*, 2000). This propensity for hybridisation is a characteristic of at least some insular groups, such as Hawaiian Compositae (see Baldwin, 1998) and Canary Island *Argyranthemum* (Brochmann *et al.*, 2000).

6.1.1 Breeding and sexual systems

Outcrossing, selfing and apomictic breeding systems occur in the Compositae (see Lane, 1996). Insects are the predominant animal pollinators (Lane, 1996), but some species are adapted for wind pollination (Berry and Calvo, 1989; Garnock-Jones, 1986; Payne, 1963). Agamospermy (the production of fertile seed without the fusion of gametes) is common in some genera, including *Antennaria*, *Crepis*, *Hieracium* and *Taraxacum* (see Grant, 1981 pp. 418–424) and cleistogamy is reported in *Centaurea melitensis* L. (Porras and Alvarez, 1999). Some species bear only hermaphrodite florets, but dicliny (modes of dioecy and monoecy) is widespread in the family. In the Compositae the hermaphrodite florets are always protandrous. The syngenesious stamens mature before the pistil and exhibit introrse dehiscence. The style elongates through the stamens and the pollen is presented on the tips or outer surface of the closed stigmatic arms. Autogamy (the transfer of self-pollen within the same flower) can occur only in hermaphrodite florets. Geitonogamy (the transfer of pollen between flowers on the same plant) is possible both within and between capitula.

Comparatively little is known with regard to the breeding system of New Zealand Gnaphalieae. Wilton (1997) studied the flowering phenology of 18 species of indigenous Gnaphalieae in central Canterbury. All except one (*Raoulia haastii*) clearly exhibited interfloral protogyny, i.e. the female florets mature before the functionally male or hermaphrodite florets within a capitulum. Wilton (1997) inferred from capitulum structure, pollen:ovule ratios, and phenological differences, that the majority of the species were outcrossers and only the two species of *Euchiton* (*E. audax* and *E. traversii*) had adaptations for inbreeding. Most New Zealand Gnaphalieae are gynomonoecious, i.e. the peripheral florets within a capitulum are female and the central florets are functionally male or hermaphrodite. Certain species, such as *Ozothamnus leptophyllus*, produce only hermaphrodite florets.

Self-incompatibility is a genetic mechanism preventing zygote formation after self-pollination in fertile hermaphrodite seed plants and is widespread amongst angiosperms (de Nettancourt, 1977). Many taxa in the Compositae are either strongly self-incompatible (SI) or highly self-compatible (e.g., Carr *et al.*, 1986; DeMauro, 1993; Messmore and Knox, 1997), but in some

Compositae the degree of SI varies within taxa or populations (Babcock, 1947; Reinartz and Les, 1994; Hiscock, 2000b; Young *et al.*, 2000). Pseudo self-compatibility (self-fertilisation in normally self-incompatible plants) might exist in some species (Verma and Singla, 1990; Hiscock, 2000a). Among native Compositae, *Pleurophyllum speciosum* Hook.f. is reported to be SI, but *P. criniferum* Hook.f. is highly self-compatible and hybrids between the two species exhibit an intermediate level of self-compatibility (Nicholls, 2000). A single plant of *Leptinella pectinata* (Hook.f.) D.G.Lloyd & C.J.Webb failed to set seed after self-pollination (Lloyd, 1972b). The Compositae are considered to possess a homomorphic sporophytic SI system (de Nettancourt, 1977 p. 17). In sporophytic systems, the incompatibility phenotype of the pollen grain is determined by the genotype of the anther (i.e., the sporophyte parent), not the genotype of the pollen grain, and incompatibility is expressed on the stigmatic surface. However, inhibition of pollen germination on the stigmatic surface is not absolute in some Compositae (Verma and Singla, 1990; Young *et al.*, 2000; Hiscock *et al.*, 2002). Many inferences of SI in the Compositae are casual, but substantive evidence has been published for some taxa (e.g., Knox, 1973; Carr *et al.*, 1986; DeMauro, 1993; Messmore and Knox, 1997; Hiscock, 2000b; Young *et al.*, 2000).

Self-compatible plants are believed to be derived from SI ancestors (Stebbins, 1957). Stebbins (1950) considered the loss of SI had occurred often in angiosperms, but few studies have reported active selection against SI in natural populations, as Reinartz and Les (1994) argue is occurring in *Aster furcatus*. Among native Compositae eleven species are reported to be self-compatible (Table 6.1). A variety of mutations affecting the expression of the SI genes and associated loci can account for self-compatibility (de Nettancourt, 1977; Nasrallah and Nasrallah, 1993). Segregation analyses in *Carthamus flavescens* Willd. (Imrie and Knowles, 1971), *Stephanomeria exigua* subsp. *coronaria* (Greene) Gottlieb and *S. malheurensis* Gottlieb (Brauner and Gottlieb, 1987) indicate self-compatibility is easily acquired in the Compositae and is determined by a single allele in these species.

Species	Reference
Cotula australis, C. coronopifolia	Lloyd (1972a)
Leptinella atrata, L. minor, L. pectinata	Lloyd (1972b)
Pleurophyllum criniferum	Nicholls (2000)
Senecio carnosulus, S. glaucophyllus, S. lautus, S. radiolatus, S. sterquilinus	Ornduff (1960; 1962)

Table 6.1. New Zealand native Compositae reported to be self-compatible. Note: both *Cotula* species may be adventive (Webb, 1988).

6.1.2 Interspecific and intergeneric incompatibility

De Nettancourt (1977 p. 141) defined interspecific incompatibility as "any of the post-pollination processes preventing, through an absence of pollen germination or an abnormal behaviour of pollen tubes, the formation of hybrid zygotes combining the genomes of two different fertile species or fertile ecotypes". Pre-zygotic interspecific incompatibility has been termed 'incongruity' by Hogenboom (1975). These barriers contribute to the reproductive isolation of populations or taxa and thus determine an 'upper limit' to outbreeding. Some authors (e.g., de Nettancourt, 1977) have suggested interactions between the self-incompatibility (*S*) locus and other loci may be responsible for interspecific incompatibility. Hogenboom (1975) considered incongruity is caused by genic disharmony between the pistil and pollen grain and is unrelated to the SI system, citing studies of anatomical, genetic and physiological differences between the two processes. De Nettancourt *et al.* (1974), for example, observed ultrastructural differences between self- and interspecific incompatibility in interspecific *Lycopersicon* Mill. crosses and concluded the two rejection processes are closely related but distinct. Incongruity may be expressed as a different phenomenon in different crosses. Post-fertilisation reproductive barriers, such as embryo abortion, hybrid weakness or hybrid sterility, were also considered manifestations of incongruity by Hogenboom (1975). In multiallelic sporophytic SI systems, reciprocal differences in cross-compatibility and cross-incompatibility between two genotypes occur when the parents share one *S* allele (Richards 1997 p. 226). In *Cosmos* Cav. interspecific incompatibility may be sporophytically determined (Knox *et al.*, 1972; Howlett *et al.*, 1975).

Many angiosperm genera contain closely related self-compatible and self-incompatible species (Lloyd, 1968). Often in crosses between such taxa, pollen from self-compatible species is inhibited on the stigma of self-incompatible species, but pollen from self-incompatible species is able to germinate and fertilise ovules of self-compatible species. This phenomenon has been termed 'unilateral incompatibility' (Lewis and Crowe, 1958) and has been recorded in at least 15 genera in a range of angiosperm families (de Nettancourt, 1977 p. 147). Lewis and Crowe (1958) hypothesised unilateral incompatibility (UI) was generally applicable to both gametophytic and sporophytic SI systems, unless a self-compatible taxon had recently evolved from a self-incompatible ancestor, and believed the *S* locus was directly involved. However, exceptions to the UI rule have been reported (e.g., Hogenboom, 1975; Sorensson and Brewbaker, 1994; Arnold and Richards, 1998) and UI can occur, for example, in crosses between two SI or or two self-compatible plants (de Nettancourt, 1977 p. 148).

Although non-reciprocity of crosses is recorded in the Compositae (e.g., Abbott and Lowe, 1996; Young *et al.*, 2000), the genetic or physiological basis has not been investigated.

6.1.3 Objectives

The primary objective was to assess relationships among the indigenous Gnaphalieae from artificial intergeneric crosses. Additional objectives were to assess, through artificial crosses, possible affinities between indigenous and exotic Gnaphalieae and to gain an indication of fertility in putative intergeneric hybrids. Other aims were to assess pollen stainability in a range of artificial and natural putative hybrids and to assess the self-compatibility of plants used in the experimental crosses.

6.2 Materials and Methods

Individual plants from 40 indigenous and 11 exotic Gnaphalieae were utilised in the artificial crosses and self-pollinations (see Table 6.2 p. 269).

6.2.1 Pollination methodology

All plants were grown in an unheated glasshouse as described in Chapter 4. Prior to and following pollination, all pollinated plants were placed in cages in the glasshouse, the tops of which were covered with insect cloth, the sides with clear polythene plastic, and the base with black polythene plastic. All pollinations were performed manually in the laboratory with an Olympus VS-IV stereo microscope (under the × 6.3 and × 10 objectives) during late morning and the afternoon. Plants were transferred between the cages and laboratory in cardboard boxes to minimise wind-mediated and mechanical movement of pollen during transport. Only freshly presented pollen and florets with style arms unblemished and free of pollen were used.

Female florets were preferentially pollinated. Occasionally unemasculated hermaphrodite florets were pollinated when the availability of female florets was limiting, but only florets lacking self-pollen on the stigma arms were used. Unpollinated hermaphrodite florets were removed from capitula prior to anthesis to ensure no self-pollen was present (Plate 13 A p. 276). Most pollinations were performed on a single day, but when floret numbers were restricted or all florets within a capitulum were to be pollinated, pollination over two or more days was often necessary as floret development within a capitulum is not synchronised (see Wilton, 1997). A dissecting needle or fine-tipped forceps were used to transfer pollen. Whenever possible florets were pollinated individually, but in some species (e.g., *Euchiton* species and *Anaphalioides* capitula with hermaphrodite florets removed) the florets were too

Genus	Species
Indigenous species	
Anaphalioides Kirp.	*A. alpina* (Cockayne) Glenny
	A. bellidioides (G.Forst.) Glenny
	A. hookeri (Allan) Anderb.
	A. trinervis (G.Forst.) Anderb.
Euchiton Cass.	*E. audax* (D.G.Drury) Holub
	E. cf. *involucratus* (G.Forst.) Holub
	E. delicatus (D.G.Drury) Holub
	E. lateralis (C.J.Webb) Breitw. & J.M.Ward
	E. limosus (D.G.Drury) Holub
	E. mackayi (Buchanan) Anderb.
	E. nitidulus (Hook.f.) Anderb.
	E. polylepis (D.G.Drury) Breitw. & J.M.Ward
	E. ruahinicus (D.G.Drury) Breitw. & J.M.Ward
	E. traversii (Hook.f.) Holub
Ewartia Beauverd	*E. sinclairii* (Hook.f.) Cheeseman
Helichrysum Mill., emend. Pers.	*H. coralloides* Hook.f.
	H. depressum (Hook.f.) Benth. & Hook.f.
	H. dimorphum Cockayne
	H. filicaule Hook.f.
	H. intermedium G.Simpson
	H. intermedium var. *tumidum* Cheeseman
	H. lanceolatum (Buchanan) Kirk
	H. parvifolium Yeo
Leucogenes Beauverd	*L. grandiceps* (Hook.f.) Beauverd
	L. leontopodium (Hook.f.) Beauverd
Pseudognaphalium Kirp.	*P. luteoalbum* (L.) Hilliard & B.L.Burtt
	P. luteoalbum var. *compactum* Kirk
Raoulia Hook.f.	*R. albosericea* Colenso
	R. apicinigra Kirk
	R. australis Hook.f.
	R. beauverdii Cockayne
	R. bryoides Hook.f.
	R. eximia Hook.f.
	R. glabra Hook.f.
	R. grandiflora Hook.f.
	R. haastii Hook.f.
	R. hookeri Allan
	R. hookeri "Coast"
	R. mammillaris Hook.f.
	R. monroi Hook.f.
	R. sp. "K"
	R. sp. "M"
	R. subsericea Hook.f.
	R. tenuicaulis Hook.f.
	R. youngii (Hook.f.) Beauverd
Exotic species	
Anaphalis DC.	*A. margaritacea* (L.) Benth. & Hook.f.
Antennaria Gaertn.	*A. dioica* (L.) Gaertn. 'Rosea'
Euchiton Cass.	*E.* sp.
Ewartia Beauverd	*E. catipes* (DC.) Beauverd
	E. meredithae (F.Muell.) Beauverd
	E. planchonii (Hook.f.) Beauverd
Gamochaeta Wedd.	*G. spicata* (Lam.) Cabrera
Leontopodium (Pers.) R.Br.	*L. palibinianum* Beauverd
	L. sp.
Ozothamnus R.Br.	*O. hookeri* Sond.
	O. rodwayi Orchard
	O. rosmarinifolius
	O. scutellifolius Hook.f.
Vellereophyton Hilliard & B.L.Burtt	*V. dealbatum* (Thunb.) Hilliard & B.L.Burtt

Table 6.2. Indigenous and exotic Gnaphalieae utilised in the artificial crosses and self-pollinations.

congested to allow this. Application of a similar pollen load (in the order of 10-15 grains per floret) was aimed for in all crosses.

Once the styles had turned brown, cotton thread was tied around each individual capitulum to prevent dispersal of the mature cypselas before the capitula could be harvested. The capitula were collected when the involucral bracts had turned brown. The cypselas were classified into three groups: enlarged cypselas containing seeds ('filled cypselas'); cypselas that had enlarged but which were shrivelled and appeared to be empty; and cypselas that had not enlarged and were empty. The level of cross-compatibility, as indicated by the percentage of filled cypselas, was categorised as summarised in Table 6.3. The number of unenlarged cypselas was often estimated rather than counted precisely. Enlarged cypselas were stored at 4°C in airtight jars containing silica gel.

Self-compatibility

Individual plants from a range of species, which were used in the experimental intergeneric crosses, were self-pollinated by hand to assess the potential for self-fertilisation. For some populations two to six individual plants were tested, but in most instances only one plant per population was available. The number of individual plants per species tested ranged from one to 16 (see Table 6.5 p. 292–294). In plants of *R. australis*, *R. hookeri*, *R. mammillaris* and R. *tenuicaulis*, the style arms were usually extremely short or rudimentary in hermaphrodite florets, making self-pollination of such florets difficult or impossible. The level of vector-independent self-pollination ('autonomous selfing') was assessed by allowing unmanipulated capitula to mature naturally within the cages without hand pollination. The presence of agamospermy was tested by removing the hermaphrodite florets from capitula prior to pollen presentation; such capitula are termed 'emasculated capitula'.

Cross-compatibility	Filled cypselas (%)
high	> 75
moderate	30–75
low	< 30

Table 6.3. Levels of cross-compatibility recognised. Classes are based on the proportion of filled cypselas in experimental pollinations.

Crosses among indigenous Gnaphalieae

Crosses among a wide range of indigenous Gnaphalieae were performed as a broad survey of cross-compatibility in the group. Performance of a complete series of reciprocal crosses was not possible, owing to a number of practical obstacles (see Discussion pp. 298 & 301). The crosses preferentially involved the following 'target' species: *Anaphalioides bellidioides*, *A. trinervis*, *Euchiton audax*, *Helichrysum intermedium*, *Helichrysum lanceolatum* and *Raoulia tenuicaulis*. Two to four crosses using plants of different provenances were performed for some species combinations, but in many cases only a single cross between two species was possible. Reciprocal crosses were performed in some combinations. *Ozothamnus leptophyllus* produces only hermaphrodite florets and the stigmas are readily contaminated with self-pollen. Logistically it was not possible to dissect out the anthers prior to their dehiscence, so to avoid the possibility of mentor effects no crosses using *O. leptophyllus* as the maternal parent were performed. The number of female florets per capitulum in other species ranges from less than five, as in *Helichrysum depressum* and *Raoulia haastii* (Wilton, 1997), to more than 200 in cultivated plants of *Euchiton traversii*. Consequently, the number of capitula used varied between crosses.

Crosses between indigenous and exotic Gnaphalieae

Artificial crosses between individual plants of indigenous and exotic species were also performed. Individual plants of ten exotic species were used (Table 6.4). Three individual plants of *E. planchonii* and single individuals of the remaining species were used in the crosses. The cross-compatibility of plants of two native species (*Ozothamnus leptophyllus* and *Pseudognaphalium luteoalbum*), which are not closely related to the other indigenous Gnaphalieae (Ward, 1993; Breitwieser *et al.*, 1999), with plants of exotic species was of particular interest. The provenance of most plants was known, except for *A. margaritacea*, *A. dioica* and *L. palibinianum*, which were obtained from local nurseries. The plants of *A. margaritacea* and *Ewartia meredithae* used were gynoecious (i.e., the hermaphrodite florets are

Exotic species	
Anaphalis margaritacea	*Leontopodium palibinianum*
Antennaria dioica	*Leontopodium* sp.
Ewartia meredithae	*Ozothamnus hookeri*
Ewartia planchonii	*Ozothamnus rosmarinifolius*
Gamochaeta spicata	*Vellereophyton dealbatum*

Table 6.4. Exotic species, of which individual plants were utilised in the artificial crosses.

functionally female). Because the hermaphrodite florets produced copious nectar, which might cover the style arms of neighbouring female florets and potentially disrupt cross-pollination, the hermaphrodite florets were removed prior to anthesis. The only plants of *A. dioica* and *L. palibinianum* available for use were androecious (i.e., female florets are absent or nonfunctional, and the hermaphrodite florets are functionally male).

Crosses involving natural putative intergeneric hybrids

To investigate their fertility, experimental crosses were also performed with single plants of the following natural putative hybrids: *A. bellidioides* × *H. lanceolatum*, *A. bellidioides* × *H. intermedium* var. *tumidum* 'Graeme Paterson', *H. dimorphum* × *H. filicaule*, *H. intermedium* × *H. lanceolatum* and *R. hectorii* × *R. subsericea*.

6.2.2 Pollen germination on the stigma

Pollen germination on the stigma was evaluated in some crosses (mainly crosses between plants of indigenous and exotic species) by staining fresh stigmas with Alexander's differential stain (Alexander, 1980). For preparation of the stain solution, see p. 91. In most crosses stigmas were collected one day after pollination, immediately placed in a drop of stain on a slide and viewed with brightfield optics using an Olympus BH2 or Leitz DIAPLAN compound microscope. In some crosses style retraction occurred within 2 h of pollination, in which case stigmas were collected 1–1.5 h after pollination (before the styles had retracted), as logistically it was difficult to dissect the corolla tube away from the retracted styles without potentially dislodging or damaging grains on the stigma. The slides were viewed within 30 minutes of mounting, in which time there is no danger of pollen tubes being obscured by staining of the stigmatic papillae. The cytoplasm of pollen grains and pollen tubes stain reddish-purple and the grain wall pale green. In order to distinguish between germinated and ungerminated grains, the style arms were not squashed after mounting. Consequently, some pollen grains were obscured and the presence or absence of a pollen tube could not be determined. Thus the method only allows comparison of the approximate germination frequency between crosses. Between six and 28 stigmas per cross were examined.

6.2.3 Style retraction after pollination

The proportion of retracted styles following pollination (see Plate 13 B p. 276) was counted. In certain species, such as *Euchiton* species, it was not possible to count individual florets because of the large number of florets and their density in the capitulum. For these species, the percentage of florets with retracted styles was estimated. Although florets with retracted styles

were usually readily distinguishable one day after pollination, capitula were examined daily for three days after pollination, as in most species the style arms of unpollinated florets gradually curl at the tips with age (see Wilton, 1997) and thus unretracted styles become more prominent.

6.2.4 Seed germination

Seeds from a random selection of crosses were sown to confirm that the hybrids were 'true to parentage' and to assess the viability of filled, enlarged cypselas produced. Seed was germinated as described on p. 88. Seedlings were pricked out into 5 cm diameter plastic pots once several true leaves had developed and were subsequently treated the same as mature plants.

6.2.5 Pollen stainability

Pollen from a number of experimental hybrids and natural putative hybrids was stained with Alexander's differential stain (as described on p. 91). Freshly presented pollen was collected from cultivated plants growing in the glasshouse. For each plant pollen was collected from three to six florets, each from separate capitula, and 200 grains were counted per floret.

6.3 Results

For brevity in all tables in this section, the provenance of each plant is denoted by a letter after the species name. See Appendix 2 for a key to the plant provenances. To avoid confusion in some sections, the following generic abbreviations are used: *Antennaria* (*Ant.*), *Euchiton* (*Eu.*), and *Ewartia* (*Ew.*).

6.3.1 Post-pollination events

Retraction of the style into the corolla tube following pollination occurred in all crosses in which enlarged cypselas developed, although it was less obvious in *Helichrysum lanceolatum* than other species studied. In most compatible crosses style retraction occurred within 24 h after pollination (Plate 13 B p. 276). However, in compatible crosses in which *Euchiton audax* was the maternal parent, the styles retracted within 90-120 min after pollination. In some crosses, such as *Raoulia tenuicaulis* × *Anaphalioides bellidioides*, the styles had only partly retracted 24 h after pollination but were fully retracted after 48 h. In many compatible crosses some styles did not retract after pollination, and in incompatible crosses most or all styles remained unretracted; in these florets the style arms gradually became necrotic and shrivelled. In some incompatible interspecific crosses (e.g., *R. tenuicaulis* F × *A. alpina* B and some crosses in which *Leucogenes leontopodium* was the maternal parent), the stigma of pollinated florets started to turn brown 1 d after pollination and the styles did not retract, whereas unpollinated stigmas remained in perfect

condition and only became necrotic after 1-2 weeks. Where an exact count of the proportion of retracted styles was possible, the proportion of enlarged cypselas was always equal to or lower than the number of retracted styles (e.g., *Raoulia tenuicaulis* C × *Anaphalioides bellidioides* B). The cypselas matured after 3-5 weeks.

6.3.2 Self-compatibility and autonomous selfing

Indigenous Gnaphalieae

Fourteen individual plants from eight species exhibited high self-compatibility (Table 6.5 pp. 292–294). All but one of these plants also showed a moderate or high level of autonomous selfing. In all plants studied seed set in emasculated, unpollinated capitula was absent or extremely low (less than 2 %).

Nine individual plants from six *Euchiton* species were strongly self-compatible and exhibited a similar level of seed set in each treatment. Seed set following self-pollination exceeded 85 % in all plants. The level of seed set from autonomous selfing was similar or slightly lower, ranging from 62 % in the *E.* cf. *involucratus* plant to 94 % in a plant of *E. traversii*. Except for three seeds from a single capitulum in one *E. audax* plant, seed set was absent in emasculated, unpollinated capitula.

Individual plants of *Anaphalioides hookeri* and *A. trinervis* were also strongly self-compatible. Seed set following selfing was 72 % in the *A. hookeri* plant and 86 % in the *A. trinervis* plant. Autonomous selfing occurred in the *A. hookeri* plant but not in *A. trinervis*. Self-pollinations demonstrated three other plants of *A. trinervis* (A, B and D) were self-compatible, but quantative data were not collected for these plants.

Two plants of *Pseudognaphalium luteoalbum* and one plant of *P. luteoalbum* var. *compactum* exhibited high self-compatibility and, in two of these plants, moderate levels of autonomous selfing. Self-pollinations showed that three other plants of *P. luteoalbum* (A, B and D/2) and two other plants of *P. luteoalbum* var. *compactum* (A/2 and A/3) were also self-compatible, but quantitative data was not collected for these plants.

All other plants tested were strongly self-incompatible and set very little or no seed following self-pollination and in unmanipulated capitula. This group comprised plants of *Anaphalioides* (*A. alpina* and *A. bellidioides*), *Ewartia sinclairii*, *Helichrysum*, *Leucogenes*, *Ozothamnus* and

Raoulia. Some germinated pollen grains were observed on the stigma after selfing in plants of *O. leptophyllus* (Plate 13 C p. 276), *R. haastii*, *R. hookeri* (Plate 13 D) and *R.* sp. "K".

Exotic Gnaphalieae

Individual plants of a Tasmanian *Euchiton* species, *Ewartia planchonii*, *Gamochaeta spicata* and *Vellereophyton dealbatum* were strongly self-compatible, setting over 75 % seed after self-pollination (Table 6.6 p. 295). The level of autonomous selfing was moderate in the plants of *G. spicata* and *V. dealbatum* and high in the *Euchiton* species and *Ew. planchonii*. A single filled cypsela in the *V. dealbatum* plant was obtained from a total of 19 emasculated, unpollinated capitula in these plants.

Plate 13. Post-pollination stages in experimental pollinations.

A, Emasculated, unpollinated *Anaphalioides bellidioides* capitulum. The hermaphrodite florets were removed prior to pollen presentation. Scale bar = 1.5 mm.

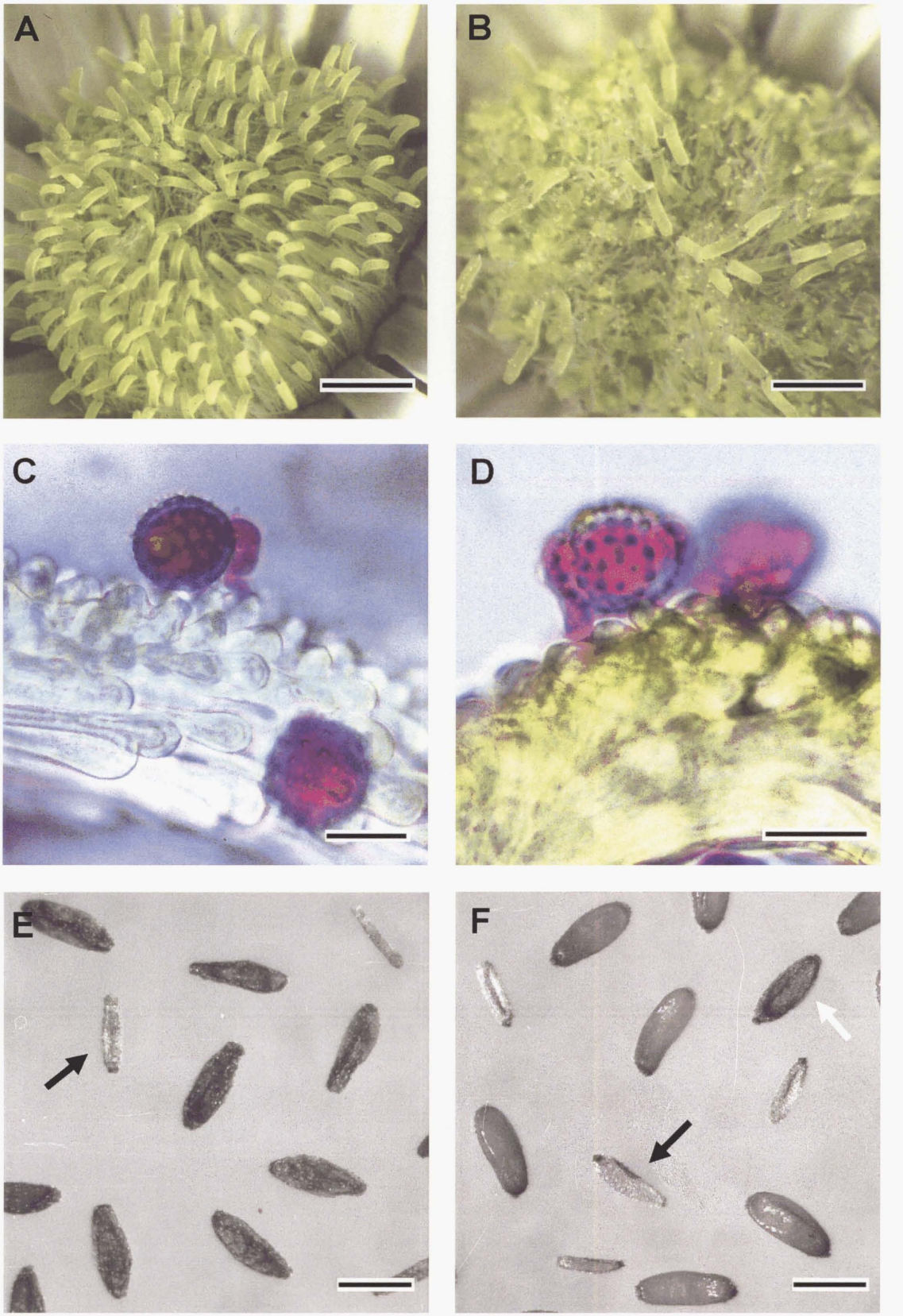
B, The same *A. bellidioides* capitulum 24 h after application of *Raoulia tenuicaulis* pollen. Note that many of the styles have retracted into the corolla tubes. Scale bar = 1.5 mm.

C, Germinated pollen grain on an *Ozothamnus leptophyllus* D stigma after self-pollination. Scale bar = 20 μm.

D, Germinated pollen grain on a *Raoulia hookeri* A stigma after self-pollination. Scale bar = 20 μm.

E, Cypselas from the cross *Euchiton audax* B × *Ozothamnus leptophyllus* D. All enlarged cypselas are empty. Arrow indicates an empty, unenlarged cypsela. Scale bar = 0.5 mm.

F, Cypselas from the cross *Gamochaeta spicata* × *Euchiton audax* B. Most enlarged cypselas are filled, but the white arrow indicates an enlarged, empty cypsela; the black arrow indicates an empty, unenlarged cypsela. Scale bar = 0.5 mm.


A
B
C
D
E
F

Plant	Individual plants pollinated	Treatment	No. of capitula	Style retraction	Filled cypselas	Unenlarged cypselas	Seed set (%)
Anaphalioides							
A. alpina A	1	A	3	-	0	c. 400	0
A. alpina B	1	A	3	-	0	c. 350	0
A. bellidioides A	1	A	3	-	0	c.350	0
A. bellidioides B	1	A	3	-	2	665	0.3
		B	3	-	0	c.650	0
A. bellidioides D, E	2	A	6	-	0	c. 550	0
A. bellidioides F	2	A	6	-	0	c. 900	0
A. bellidioides G	2	A	6	-	0	c. 700	0
A. bellidioides H	1	A	3	-	0	c. 450	0
A. bellidioides I	6	A	18	-	1	c. 2150	c. 0.05
A. bellidioides J	2	A	6	-	0	c. 700	0
A. hookeri	1	A	3	+	527	209	71.6
		B	1	+	114	90	55.9
		C	3	-	0	c. 800	0
A. trinervis C	1	A	5	+	935	152	86
		B	5	+	6	1211	0.5
		C	1	+	2	131	1.5
Euchiton							
E. audax A	1	A	3	+	188	21	90
		B	1	+	43	4	91.5
		C	3	-	3	c. 450	c. 0.7
E. audax B	1	A	6	+	330	53	86.2
		B	3	+	163	60	73.1
		C	12	-	0	771	0
E. cf. *involucratus*	1	A	4	+	131	20	86.8
		B	5	+	196	122	61.6
		C	5	-	0	330	0
E. mackayi	1	A	2	+	154	12	92.8
		B	2	+	118	11	91.5
E. ruahinicus	1	A	6	+	317	18	94.6
		C	5	-	0	c. 250	0
E. traversii A	1	A	2	+	571	55	91.2
		B	5	+	959	175	84.6

Table 6.5. Self-compatibility of plants of indigenous Gnaphalieae. See Appendix 2 for plant provenances. (continued overleaf)

Treatments: A, all florets self-pollinated by hand; B, autonomous selfing (no florets hand pollinated); C, capitula emasculated (hermaphrodite florets removed prior to pollen presentation) but female florets not hand pollinated. **Style retraction**: +, present; -, absent.

Table 6.5 (continued).

Species / locality	Individual plants pollinated	Treatment	No. of capitula	Style retraction	Filled cypselas	Unenlarged cypselas	Seed set (%)
Euchiton							
E. traversii B/1	1	A	3	+	865	128	87.1
		B	3	+	720	139	83.8
		C	4	-	0	c. 1000	0
E. traversii B/2	1	A	4	+	1050	39	96.4
		B	4	+	960	95	91
		C	3	-	0	c. 750	0
E. traversii C	1	A	5	+	994	80	92.6
		B	4	+	1053	73	93.5
		C	4	-	0	c. 1200	0
Ewartia							
E. sinclairii A	4	A	24	-	0	c. 600	0
E. sinclairii B	1	A	6	-	0	c. 300	0
Helichrysum							
H. depressum	1	A	6	-	0	c. 70	0
H. filicaule A	1	A	6	-	0	c. 200	0
H. filicaule B	1	A	6	-	0	c. 200	0
H. filicaule C	1	A	6	-	0	c. 200	0
H. intermedium A	1	A	6	-	0	c. 450	0
H. intermedium B	1	A	8	-	0	c.650	0
		B	8	-	0	685	0
H. intermedium D	1	A	6	-	0	c. 300	0
Leucogenes							
L. leontopodium A	1	A	3	-	0	c. 120	0
		B	7	-	0	302	0
Ozothamnus							
O. leptophyllus A	1	A	8	-	0	c. 80	0
O. leptophyllus D	1	A	15	-	0	c. 165	0
Pseudognaphalium							
P. luteoalbum C	1	A	2	+	125	24	83.9
P. luteoalbum D/1	1	A	3	+	285	21	93.1
		B	2	+	73	166	30.5
		C	3	-	0	c. 330	0
P. luteoalbum var. *compactum* A/1	1	A	3	+	445	28	94.1
		B	4	+	290	185	61.1
		C	1	-	0	112	0
Raoulia							
R. albosericea A	1	A	6		0	c. 120	0
R. albosericea B	1	A	6		0	c. 120	0
		B	10		0	c. 200	0

Table 6.5 (continued).

Species / locality	No. of plants pollinated	Treatment	No. of capitula	Style retraction	Filled cypselas	Unenlarged cypselas	Seed set (%)
Raoulia							
R. apicinigra D	1	A	6		0	c. 150	0
		B	8		0	c. 150	0
R. australis B	2	A	20		0	c. 400	0
R. australis C	1	A	10		0	c. 200	0
R. australis D	1	A	10		0	c. 200	0
R. beauverdii A	1	A	9	-	0	c. 110	0
		B	10	-	0	c.120	0
R. beauverdii B	1	A	4	-	0	c. 50	0
R. beauverdii C	1	A	3	-	0	30	0
R. bryoides A	1	A	6		0	c. 70	0
R. eximia	6	A	18		0	c. 280	0
R. glabra B	1	A	6		0	c. 120	0
R. grandiflora B	1	A	6		0	c. 120	0
R. grandiflora C	1	A	6		0	c. 120	0
R. haastii A/3	1	A	10	-	0	40	0
		B	18	-	0	73	0
R. hookeri A	1	A	6		0	c. 120	0
		B	34		0	c. 650	0
R. hookeri C	1	A	6		0	c. 120	0
R. hookeri D	1	A	6	-	0	c. 120	0
R. hookeri E	1	A	6	-	0	c. 120	0
R. hookeri "Coast"	1	A	6	-	0	c. 120	0
R. mammillaris	1	A	11	-	0	c. 140	0
R. monroi B	1	A	6	-	0	c. 70	0
R. monroi C	1	A	6	-	0	68	0
R. sp. "K"	1	A	6	-	0	c. 80	0
		B	10	-	0	c. 120	0
R. subsericea A	1	A	6	-	0	c. 100	0
R. subsericea B	1	A	4	-	0	c. 60	0
		B	3	-	0	44	0
R. subsericea C	1	A	6	-	0	c. 100	0
		B	10	-	0	c. 150	0
R. subsericea D	1	A	6	-	0	c. 100	0
R. tenuicaulis A	1	A	8	-	0	36	0
R. tenuicaulis B	1	A	8	-	0	45	0
R. tenuicaulis C	1	A	11	-	0	64	0
R. tenuicaulis D	1	A	7	-	0	39	0
R. tenuicaulis E	1	A	11	-	0	87	0
		B	17	-	1	136	0.7
R. tenuicaulis F	1	A	6	-	0	30	0

Species / locality	No. of plants pollinated	Treatment	No. of capitula	Style retraction	Filled cypselas	Unenlarged cypselas	Seed set (%)
Euchiton sp.	1	A	4	+	147	18	89.1
		B	6	+	236	17	93.3
		C	6	-	0	c. 240	0
Ewartia planchonii B	1	A			234	35	87
		B	4	+	192	63	75.3
		C	4	-	0	347	0
Ewartia planchonii C/3	1	A	3	+	288	37	88.6
		B	3	+	253	41	86.1
Gamochaeta spicata	1	A	5	+	265	82	76.4
		B	6	+	238	193	55.2
		C	6	-	0	c. 360	0
Vellereophyton dealbatum	1	A	6	+	107	9	92.2
		B	2	+	15	20	42.9
		C	3	-	1	c. 360	c. 0.3

Table 6.6. Self-compatibility of plants of exotic Gnaphalieae. See Appendix 2 for plant provenances.

Treatments: A, all florets self-pollinated; B, autonomous selfing (no florets self-pollinated); C, capitula emasculated (hermaphrodite florets removed prior to pollen presentation) but female florets not self-pollinated. **Style retraction**: +, present; -, absent.

6.3.3 Experimental crosses among indigenous Gnaphalieae

The results of all artificial crosses performed are summarised in Tables 6.7–6.20 (pp. 285–297). Note that for crosses between two 'target species', the results are duplicated in the summary tables for *both* target species, in order to consolidate *all* crosses performed with a particular 'target species' in a single table.

Anaphalioides bellidioides

Plants of *A. bellidioides* were cross-compatible with plants of 15 species from five genera (Table 6.7 p. 285). Filled cypselas were obtained in 24 intergeneric crosses with plants of six *Euchiton* species (six crosses), *Ewartia sinclairii* (two crosses), *Leucogenes leontopodium* (four crosses), whipcord *Helichrysum* species (five crosses), *H. filicaule* (one cross), *H. lanceolatum* (two crosses) and four *Raoulia* species (four crosses). Plants of *A. bellidioides* were moderately or highly cross-compatible with plants of *Euchiton audax*, *Eu. delicatus*, *Eu.* cf. *involucratus*, *Ew. sinclairii*, *H. intermedium*, *Raoulia haastii* and *R. tenuicaulis*. Reciprocal crosses between the same individual plants were performed in seven instances; marked differences in the level of cross-compatibility were expressed in five of these crosses, e.g. in reciprocal crosses between *A. bellidioides* I and *Ew. sinclairii* B. Crosses between plants of the same species from different provenances yielded differences in cross-compatibility, e.g., crosses with *R. tenuicaulis* (based on three crosses), *H. intermedium* (based on two crosses) and *L. leontopodium* (based on four crosses). In most crosses, empty enlarged cypselas were absent or rare. Two exceptions were the crosses *A. bellidioides* H × *L. leontopodium* A and *R. tenuicaulis* C × *A. bellidioides* B, in which over 40 % of the cypselas had enlarged but were empty. In general, estimates of style retraction were consistent with or exceeded the proportion of enlarged cypselas in individual crosses. Where exact counts were possible, as in the crosses *H. intermedium* B × *A. bellidioides* C and *R. tenuicaulis* C × *A. bellidioides* B, the number of retracted styles slightly exceeded the number of enlarged cypselas.

Anaphalioides trinervis

Filled cypselas were obtained in 14 intergeneric crosses (Table 6.8 p. 286). Plants of *A. trinervis* were cross-compatible with plants of seven species from five genera. Most crosses performed were incompatible or exhibited low compatibility. A reciprocal cross between plants of *A. trinervis* and *Helichrysum intermedium* was moderately to highly compatible, depending on which plant was the maternal parent. Plants of *A. trinervis* exhibited low cros-compatibility with plants of *Ewartia sinclairii* (one cross) and *Raoulia haastii* (three crosses). Plants of *A. trinervis* and *R. tenuicaulis* varied from incompatible to moderately compatible (based on seven crosses);

seed set was more frequent when the *A. trinervis* plant was the maternal parent. Single crosses with plants of *Euchiton audax*, *H. lanceolatum*, *Leucogenes grandiceps* and *L. leontopodium* also yielded filled cypselas. In a single cross between plants of *A. trinervis* and *A. alpina*, nearly 70 % of the cypselas had enlarged but were flattened, shrivelled and empty. In all other crosses involving a plant of *A. trinervis*, enlarged empty cypselas were rare or absent.

Euchiton audax

Twenty-eight crosses were performed, most of which employed as the maternal parent one of two *E. audax* plants originating from the Volcanic Plateau. Intergeneric crosses were compatible with plants of *Anaphalioides* (two crosses), *Ewartia sinclairii* (one cross), *Helichrysum* (four crosses), *Leucogenes* (one cross) and *Raoulia* subg. *Raoulia* (nine crosses) (Table 6.9 p. 287). Of the 17 intergeneric crosses that yielded filled cypselas, cross-compatibility was moderate or high in 13 crosses. An interspecific cross (*Eu. audax* A × *Eu. traversii* A) yielded the highest cross-compatibility (95 % seed set). Single crosses with plants of *A. bellidioides* and *H. lanceolatum* exhibited high cross-compatibility. Enlarged empty cypselas, but no filled cypselas, were obtained in crosses with plants of *Ozothamnus leptophyllus* (two crosses), *Pseudognaphalium luteoalbum* (five crosses) and in single crosses with plants of *Raoulia bryoides*, *R. hookeri* and *R. subsericea*. Estimates of style retraction following pollination were consistent with the proportion of enlarged cypselas in individual crosses.

Helichrysum intermedium

Intergeneric crosses with plants of 14 species from five genera yielded filled cypselas (Table 6.10 p. 288). Plants of *H. intermedium* were cross-compatible with plants of four *Anaphalioides* species (nine crosses), two *Euchiton* species (three crosses), *H. lanceolatum* (one cross), two *Leucogenes* species (three crosses) and five *Raoulia* species (12 crosses). The level of cross-compatibility was high in most of the 20 compatible crosses. Cross-compatibility with different plants of *Anaphalioides bellidioides* (four crosses) and *Raoulia apicinigra* (three crosses) varied from incompatible to highly compatible. Differences in cross-compatibility occurred in reciprocal crosses with plants of *A. alpina*, *A. bellidioides*, *E. audax*, *L. leontopodium*, *R. apicinigra*, *R. beauverdii* and *R.* sp. "M". In most crosses enlarged, empty cypselas were absent or rare. A notable exception was the cross *H. intermedium* A × *R. grandiflora* Mt Hutt, in which the styles retracted in 37 out of 40 pollinated florets. The cypselas enlarged in all of these florets but all were empty. Estimates of style retraction were generally consistent with or exceeded the proportion of enlarged cypselas. In some crosses, notably with plants of *Anaphalioides* and *Leucogenes* species, the proportion of retracted styles was extremely high.

Helichrysum lanceolatum

Filled cypselas were produced in crosses with plants of 10 species from four genera (Table 6.11 p. 289). *H. lanceolatum* was compatible with four *Anaphalioides* species (six crosses), *Euchiton audax* (two crosses) and in single crosses with *E. limosus*, *E. traversii*, *H. intermedium*, *Raoulia monroi* and *R. tenuicaulis*. Reciprocal differences in cross-compatibility were suggested from a single reciprocal cross with a plant of *A. bellidioides* and from single reciprocal crosses (but using different individuals) with plants of *A. hookeri* and *E. audax*. Most crosses were of low to moderate compatibility; only single crosses with *E. audax* and *R. tenuicaulis* resulted in high cross-compatibility. Enlarged, empty cypselas were rare or absent in all crosses.

Leucogenes leontopodium

Intergeneric crosses with plants of nine species from four genera yielded filled cypselas (Table 6.12 p. 289). Plants of *L. leontopodium* were cross-compatible with two *Anaphalioides* species (eight crosses), five *Euchiton* species (six crosses), *Helichrysum intermedium* (three crosses) and *Raoulia grandiflora* (two crosses). Cross-compatibility was low to moderate in most crosses; high cross-compatibility was obtained in single crosses with two *Euchiton* species only. Some variation occurred in the cross-compatibility of different plants of *A. bellidioides* and *L. leontopodium*. Reciprocal differences in cross-compatibility was suggested in crosses with plants of *H. intermedium* and *R. grandiflora*. One cross between plants of *A. bellidioides* and *L. leontopodium* was notable for the relatively high frequency (41 %) of enlarged, empty cypselas, which were absent or rare in the other crosses.

Raoulia tenuicaulis

Filled cypselas were obtained in crosses with plants of *Anaphalioides bellidioides* (four crosses), *A. trinervis* (seven crosses), three *Euchiton* species (three crosses) and *Helichrysum lanceolatum* (two crosses) (Table 6.13 p. 290). Seed set was more frequent when *R. tenuicaulis* was the paternal parent (nine such crosses performed); crosses in which a plant of *R. tenuicaulis* was the maternal parent were incompatible or of low cross-compatibility (based on 15 crosses). Overall, most crosses were incompatible or of low compatibility; moderate or high cross-compatibility was obtained in single crosses with plants of *A. bellidioides*, *A. trinervis*, *E. delicatus* and *H. lanceolatum*. Reciprocal differences in cross-compatibility were suggested from reciprocal crosses with plants of *A. bellidioides* and *A. trinervis*. In addition, the cross-compatibility between different plants of *A. bellidioides*, *A. trinervis* and *R. tenuicaulis* varied. Enlarged, empty cypselas were absent or rare in most crosses.

Other indigenous Gnaphalieae

Filled cypselas were obtained from 14 intergeneric crosses employing other indigenous Gnaphalieae (Table 6.14 p. 291). Seven crosses with plants of *Ewartia sinclairii* were performed, all of which yielded filled cypselas. Single plants of *Ew. sinclairii* and *Anaphalioides bellidioides* exhibited high cross-compatibility when *Ew. sinclairii* was the maternal parent, but low cross-compatibility in the reciprocal cross. In a single cross, plants of *Ew. sinclairii* and *Euchiton audax* were of moderate cross-compatibility. Plants of *Ew. sinclairii* exhibited low cross-compatibility with plants of *A. trinervis*, *Eu. traversii* and *Helichrysum intermedium* (based on single crosses). In a single cross between plants of *Ew. sinclairii* and *Ozothamnus leptophyllus* the styles retracted in 27 florets, but only a single filled cypsela and 18 enlarged, empty cypselas were obtained.

Plants of *Euchiton limosus* and *H. filicaule* exhibited low cross-compatibility when *E. limosus* was the maternal parent, but the reciprocal cross was incompatible. Filled cypselas were produced in the following single crosses: *Eu. nitidulus* × *Raoulia* sp. "M", *H. parvifolium* × *R. subsericea* and *Leucogenes grandiceps* × *R. eximia*. Single seeds were obtained from the crosses *A. alpina* × *R. haastii*, *H. filicaule* × *H. dimorphum* and *H. filicaule* × *R. glabra*. The seed from the former cross germinated but died at the cotyledon stage, but the seeds from the latter two crosses germinated and normal, healthy seedlings developed. In three crosses (*A. alpina* × *H. intermedium*, *Eu.* cf. *involucratus* × *L. grandiceps* and *Eu.* cf. *involucratus* × *R. hookeri*) enlarged cypselas were produced but all were empty.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
A. bellidioides I × *E. traversii* A	17/10/97	1	all ♀	0%	0	0	123	0
A. bellidioides I × *E. sinclairii* B	14/10/97	3	all ♀	ND	69	0	288	19
A. bellidioides H × *H. intermedium* C	29/10/96	1	all ♀	ND	104	0	31	77
A. bellidioides E × *H. intermedium* B	7/11/96	3	all ♀	90%	5	0	256	2
A. bellidioides F × *H. lanceolatum* B/1	24/10/96	1	all ♀	10%	12	1	184	6
A. bellidioides D × *L. leontopodium* A	18/10/96	1	all ♀	ND	23	0	69	25
A. bellidioides D × *L. leontopodium* B	22/10/96	1	all ♀	ND	7	0	74	9
A. bellidioides H × *L. leontopodium* A	27/10/96	3	all ♀	90%	179	212	124	35
A. bellidioides H × *L. leontopodium* B	28/10/96	3	all ♀	ND	113	13	237	31
A. bellidioides E × *R. beauverdii* A	8/11/96	2	all ♀	0%	0	0	169	0
A. bellidioides D × *R. grandiflora* B	18/10/96	1	all ♀	0%	0	0	108	0
A. bellidioides B × *R. haastii* A/2	25/09/96	5	all ♀	90%	390	0	183	68
A. bellidioides B × *R. monroi* C/3	27/09/96	2	all ♀	25%	60	0	198	30
A. bellidioides B × *R. tenuicaulis* C	22/09/96	3	all ♀	75%	266	0	102	72
A. bellidioides I × *R. youngii*	2/11/97	2	all ♀	0%	0	0	251	0
A. hookeri A × *A. bellidioides* A	22/10/96	5	all ♀	ND	611	0	487	56
A. hookeri A × *A. bellidioides* G/5	19/10/95	1	all ♀	ND	115	0	126	48
E. audax A × *A. bellidioides* H	22/10/96	5	all ♀	90%	317	0	67	83
E. delicatus × *A. bellidioides* H	23/10/96	4	all ♀	90%	185	0	52	78
E. cf. *involucratus* × *A. bellidioides* C	17/11/96	2	all ♀	90%	71	0	34	68
E. lateralis × *A. bellidioides* C	17/11/96	1	all ♀	10%	11	1	36	23
E. limosus A × *A. bellidioides* J	24/12/96	2	all ♀	33%	58	16	61	43
E. traversii B × *A. bellidioides* H	22/10/96	3	all ♀	90%	420	0	503	46
E. sinclairii B × *A. bellidioides* I	14/10/97	9	all ♀	90%	66	3	5	89
H. depressum × *A. bellidioides* J	19/12/96	2	2 ♀, 13 ☿	10	0	1	14	0
H. filicaule D × *A. bellidioides* J	19/12/96	1	all	0%	1	1	53	2
H. intermedium B × *A. bellidioides* E	7/11/96	3	77 ♀, 1 ☿	75%	50	14	14	64
H. intermedium B × *A. bellidioides* C	15/11/96	1	28 ♀	21	13	0	15	46
H. lanceolatum B/2 × *A. bellidioides* F	24/10/96	6	14 ♀, 47 ☿	ND	28	0	33	46
H. parvifolium × *A. bellidioides* J	21/12/96	2	11 ♀, 2 ☿	ND	1	3	9	8
L. leontopodium B × *A. bellidioides* H	28/10/96	2	18 ♀	0	0	0	18	0
R. beauverdii A × *A. bellidioides* E	9/11/96	4	29 ♀	0	0	0	29	0
R. grandiflora B × *A. bellidioides* D	20/10/96	1	14 ♀, 5 ☿	0	0	0	19	0
R. monroi C/3 × *A. bellidioides* B	23/09/96	5	27 ♀, 51 ☿	0	0	0	78	0
R. sp. "M" × *A. bellidioides* C	17/11/96	1	10 ♀, 5 ☿	0	0	0	15	0
R. tenuicaulis E × *A. bellidioides* G/3	13/10/95	3	14 ♀	ND	0	9	5	0
R. tenuicaulis E × *A. bellidioides* G/5	11/10/95	8	40 ♀	ND	0	19	21	0
R. tenuicaulis C × *A. bellidioides* B	21/09/96	12	70 ♀	44	15	19	36	21

Table 6.7. Experimental crosses with plants of *Anaphalioides bellidioides*. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ☿, hermaphrodite florets.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
A. trinervis B × *A. alpina* B	4/11/96	3	all ♀	90%	0	174	67	0
A. trinervis D × *E. audax* B	5/12/97	2	all ♀	0	0	0	c.300	0
A. trinervis B × *E. sinclairii* C	31/10/96	1	all ♀	66%	4	1	167	2
A. trinervis B × *H. intermedium* B	4/11/96	3	all ♀	90%	429	0	105	80
A. trinervis B × *L. grandiceps* B	15/11/96	1	all ♀	ND	81	14	123	37
A. trinervis B × *L. leontopodium* A	31/10/96	3	all ♀	75%	107	8	456	19
A. trinervis A × *L. leontopodium* A	22/10/96	3	all ♀	10%	0	0	265	0
A. trinervis D × *O. leptophyllus* D	5/12/97	3	all ♀	<10%	0	0	c.450	0
A. trinervis D × *P. luteoalbum* C	2/12/97	2	all ♀	0	0	0	c.300	0
A. trinervis B × *P. luteoalbum* D/2	21/11/96	2	all ♀	0	0	0	244	0
A. trinervis B × *R. haastii* A/1	29/09/96	1	all ♀	ND	4	0	192	2
A. trinervis B × *R. haastii* A/3	29/09/96	2	all ♀	ND	6	3	357	2
A. trinervis A × *R. haastii* A/2	25/09/96	5	all ♀	ND	79	0	344	19
A. trinervis B × *R. monroi* C/3	1/10/96	1	all ♀	ND	0	0	201	0
A. trinervis A × *R. tenuicaulis* C	19/09/96	5	all ♀	90%	270	0	131	67
A. trinervis B × *R. tenuicaulis* B	23/09/96	4	all ♀	90%	75	0	402	16
A. trinervis B × *R. tenuicaulis* F	1/10/96	3	all ♀	ND	15	0	619	2
E. audax B × *A. trinervis* D	21/11/96	4	all ♀	66%	146	2	116	55
H. intermedium B × *A. trinervis* B	4/11/96	3	85 ♀	100%	49	0	36	58
H. lanceolatum B/1 × *A. trinervis* B	24/10/96	13	26 ♀, 84 ♂	ND	40	0	70	36
R. tenuicaulis C × *A. trinervis* A	21/09/96	11	67 ♀	26	4	10	53	6
R. tenuicaulis C × *A. trinervis* B	29/09/96	6	35 ♀	ND	0	0	35	0
R. tenuicaulis B × *A. trinervis* B	23/09/96	9	52 ♀	8	0	0	52	0
R. tenuicaulis F × *A. trinervis* B	4/10/96	8	40 ♀	ND	0	0	40	0

Table 6.8. Experimental crosses with plants of *Anaphalioides trinervis*. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ♂, hermaphrodite florets.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlaregd cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
E. audax A × *A. bellidioides* H	22/10/96	5	all ♀	90%	317	0	67	83
E. audax B × *A. trinervis* B	21/11/96	4	all ♀	66%	146	2	116	55
E. audax A × *E. sinclairii* B	17/10/97	4	all ♀	75%	166	2	99	62
E. audax A × *E. traversii* A	17/10/97	3	all ♀	100%	193	0	11	95
E. audax B × *H. depressum*	21/12/96	2	all ♀	20%	40	1	68	37
E. audax B × *H. intermedium* B	14/11/96	6	all ♀	75%	321	32	97	71
E. audax A × *H. lanceolatum* B/1	24/10/96	4	all ♀	90%	203	0	62	77
E. audax B × *H. parvifolium*	24/12/96	2	all ♀	20%	20	6	105	15
E. audax A × *L. leontopodium* A	27/10/96	2	all ♀	10%	11	19	132	7
E. audax B × *O. leptophyllus* B	24/12/96	3	all ♀	50%	0	102	42	0
E. audax B × *O. leptophyllus* C	26/12/96	3	all ♀	90%	0	159	25	0
E. audax B × *O. leptophyllus* D	7/12/97	2	all ♀	75%	0	101	50	0
E. audax B × *P. luteoalbum* C	25/11/97	6	all ♀	75%	0	219	91	0
E. audax B × *P. luteoalbum* D/1	18/11/96	3	all ♀	50%	0	148	96	0
E. audax B × *P. luteoalbum* var. *compactum* A/3	30/11/96	4	all ♀	100%	0	186	77	0
E. audax B × *R. albosericea* B	21/12/96	3	all ♀	10%	14?	5	162	8
E. audax B × *R. apicinigra* A	24/11/96	2	all ♀	33%	73	5	59	53
E. audax B × *R. australis* A	21/11/96	5	all ♀	50%	256	9	111	68
E. audax A × *R. beauverdii* A	30/10/97	6	all ♀	50%	286	80	152	55
E. audax A × *R. bryoides* B	5/12/97	2	all ♀	100%	0	16	124	0
E. audax A × *R. haastii* A/5	17/10/97	4	all ♀	50%	95	0	129	42
E. audax B × *R. hookeri* B	24/11/96	2	all ♀	33%	0	53	72	0
E. audax B × *R. hookeri* "Coast"	11/12/96	5	all ♀	75%	1	184	71	0.3
E. audax B × *R. monroi* A	24/11/96	2	all ♀	0	3	0	108	3
E. audax A × *R. monroi* C	17/10/97	4	all ♀	50%	165	0	108	60
E. audax B × *R. subsericea* B	30/11/96	2	all ♀	75%	0	36	86	0
E. audax B × *R. tenuicaulis* G	30/11/96	2	all ♀	90%	120	3	121	49
H. intermedium B × *E. audax* B	14/11/96	2	all ♀	0	0	0	39	0
H. lanceolatum B/1 × *E. audax* C	4/11/96	4	9 ♀, 22 ♀	0	0	0	31	0
P. luteoalbum C × *E. audax* B	26/11/97	3	all ♀	0	0	15	c.330	0
P. luteoalbum D/1 × *E. audax* B	1/12/96	1	all ♀	0	0	1	102	0

Table 6.9. Experimental crosses with plants of *Euchiton audax*. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ⚥, hermaphrodite florets.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlaregd cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
H. intermedium B × *A. alpina* B	2/11/96	5	106 ♀	106	61	13	12	58
H. intermedium B × *A. bellidioides* E	7/11/96	3	77 ♀, 1 ⚥	ND	50	14	14	64
H. intermedium B × *A. bellidioides* C	15/11/96	1	28 ♀	21	13	0	15	46
H. intermedium B × *A. hookeri*	8/11/96	3	72 ♀	72	56	4	12	78
H. intermedium B × *A. trinervis* B	5/11/96	3	85 ♀	85	49	0	36	58
H. intermedium B × *E. audax* B	14/11/96	2	39 ♀	0	0	0	39	0
H. intermedium B × *E. sinclairii* C	11/11/96	1	18 ♀	ND	1	0	17	6
H. intermedium D × *H. intermedium* var. *tumidum*	23/11/95	1	8 ♀	ND	8	0	0	100
H. intermedium A × *L. grandiceps* B	13/11/96	2	42 ♀	ND	27	6	9	64
H. intermedium B × *L. leontopodium* A	5/11/96	4	97 ♀	97	63	1	33	65
H. intermedium B × *L. leontopodium* B	5/11/96	5	115 ♀	115	59	0	56	51
H. intermedium A × *R. apicinigra* C	13/11/96	1	21 ♀	20	20	0	1	95
H. intermedium D × *R. apicinigra* C	21/11/96	1	8 ♀	0	0	0	8	0
H. intermedium A × *R. australis* A	16/11/96	4	77 ♀	ND	7	0	70	9
H. intermedium B × *R. beauverdii* A	9/11/96	3	80 ♀	57	33	2	45	41
H. intermedium A × *R. grandiflora* C	13/11/96	2	40 ♀	37	0	37	3	0
H. intermedium D × *R. hookeri* B	22/11/96	4	34 ♀	0	0	0	34	0
H. intermedium D × *R. monroi* C	7/12/95	1	10 ♀	ND	2	0	8	20
H. intermedium B × *R.* sp. " M"	14/11/96	1	39 ♀	ND	15	0	24	38
H. intermedium D × *R. subsericea* B	28/11/96	4	35 ♀	ND	0	0	35	0
A. alpina B × *H. intermedium* B	10/11/96	1	all ♀	0	0	4	127	0
A. bellidioides H × *H. intermedium* C	29/10/96	1	all ♀	ND	104	0	31	77
A. bellidioides E × *H. intermedium* B	7/11/96	3	all ♀	90%	5	0	256	2
A. trinervis B × *H. intermedium* B	4/11/96	3	all ♀	75%	429	0	105	80
E. audax B × *H. intermedium* B	14/11/96	6	all ♀	75%	321	32	97	71
E. cf. *involucratus* × *H. intermedium* B	17/11/96	2	all ♀	90%	223	1	138	62
H. lanceolatum B/1 × *H. intermedium* C	27/10/96	2	5 ♀, 12 ⚥	ND	10	0	7	59
L. leontopodium B × *H. intermedium* B	7/11/96	3	18 ♀, 56 ⚥	ND	0	0	74	0
R. apicinigra C × *H. intermedium* A	27/11/96	4	2 ♀, 27 ⚥	0	0	0	29	0
R. beauverdii A × *H. intermedium* B	9/11/96	6	49 ♀	0	0	0	49	0
R. sp. "M" × *H. intermedium* B	11/11/96	1	14 ♀, 5 ⚥	0	0	0	14	0

Table 6.10. Experimental crosses with plants of *Helichrysum intermedium*. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ⚥, hermaphrodite florets.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlaregd cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
H. lanceolatum B/1 × *A. alpina* B	2/11/96	4	11 ♀, 33 ☿	0	1	0	44	2
H. lanceolatum B/1 × *A. bellidioides* F	24/10/96	6	14 ♀, 47 ☿	ND	28	0	33	46
H. lanceolatum B/1 × *A. hookeri*	24/10/96	9	18 ♀, 51 ☿	ND	24	0	45	35
H. lanceolatum B/1 × *A. trinervis* B	24/10/96	13	26 ♀, 84 ☿	ND	40	0	70	36
H. lanceolatum B/1 × *E. audax* C	4/11/96	4	9 ♀, 22 ☿	ND	0	0	31	0
H. lanceolatum B/1 × *H. intermedium* C	27/10/96	2	5 ♀, 12 ☿	ND	10	0	7	59
H. lanceolatum B/1 × *L. leontopodium* A	3/11/96	2	5 ♀, 16 ☿	ND	0	0	21	0
H. lanceolatum B/1 × *O. leptophyllus* C	4/11/96	7	16 ♀, 38 ☿	ND	0	1	53	0
H. lanceolatum B/1 × *R. monroi* B	25/10/96	7	16 ♀, 38 ☿	ND	14	0	40	26
H. lanceolatum B/1 × *R. tenuicaulis* F	4/10/96	12	24 ♀	ND	18	1	5	75
A. bellidioides F × *H. lanceolatum* B/1	24/10/96	1	all ♀	10%	12	1	184	6
A. hookeri × *H. lanceolatum* A	19/10/95	1	all ♀	ND	4	0	229	2
E. audax A × *H. lanceolatum* B/1	24/10/96	4	all ♀	90%	203	0	62	77
E. limosus A × *H. lanceolatum* C	19/12/96	2	all ♀	75%	37	1	45	45
E. traversii B × *H. lanceolatum* B/1	28/10/96	1	all ♀	0	7	0	312	2
R. tenuicaulis E × *H. lanceolatum* A	19/10/95	5	24 ♀	ND	0	6	18	0

Table 6.11. Experimental crosses with plants of *Helichrysum lanceolatum*. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ☿, hermaphrodite florets.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlaregd cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
L. leontopodium B × *A. alpina* B	5/11/96	10	all ♀	0	0	0	112	0
L. leontopodium B × *A. bellidioides* H	28/10/96	2	18 ♀	0	0	0	18	0
L. leontopodium B × *H. intermedium* B	7/11/96	3	18 ♀, 56 ☿	0	0	0	74	0
L. leontopodium B × *L. leontopodium* A	18/10/96	2	22 ♀, 10 ☿	ND	24	1	7	75
L. leontopodium B × *R. grandiflora* C	28/10/96	4	40 ♀, 9 ☿	ND	4	6	39	8
A. bellidioides D × *L. leontopodium* A	18/10/96	1	all ♀	ND	23	0	69	25
A. bellidioides D × *L. leontopodium* B	22/10/96	1	all ♀	ND	7	0	74	9
A. bellidioides H × *L. leontopodium* A	27/10/96	3	all ♀	90%	179	212	124	35
A. bellidioides H × *L. leontopodium* B	28/10/96	3	all ♀	ND	113	13	237	31
A. trinervis B × *L. leontopodium* A	31/10/96	3	all ♀	75%	107	8	456	19
A. trinervis A × *L. leontopodium* A	22/10/96	3	all ♀	10%	0	0	265	0
E. audax A × *L. leontopodium* A	27/10/96	2	all ♀	10%	11	19	132	7
E. delicatus × *L. leontopodium* A	8/11/96	1	all ♀	100%	43	0	14	75
E. cf. *involucratus* × *L. leontopodium* A	5/11/96	7	all ♀	90%	357	0	126	74
E. nitidulus B × *L. leontopodium* A	2/11/96	1	all ♀	25%	11	0	66	14
E. nitidulus A × *L. leontopodium* B	20/11/96	1	all ♀	75%	21	6	51	27
E. traversii B × *L. leontopodium* A	30/10/96	2	all ♀	33%	132	20	492	20
H. intermedium B × *L. leontopodium* A	5/11/96	4	97 ♀	97	63	1	33	65
H. intermedium B × *L. leontopodium* B	5/11/96	5	115 ♀	115	59	0	56	51
H. lanceolatum B/1 × *L. leontopodium* A	3/11/96	2	5 ♀, 16 ☿	ND	0	0	21	0
R. grandiflora A × *L. leontopodium* A	20/10/96	3	55 ♀, 27 ☿	0	0	0	82	0

Table 6.12. Experimental crosses with plants of *Leucogenes leontopodium*. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ☿, hermaphrodite florets.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Style retraction	Filled enlaregd cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		capitula	florets					
R. tenuicaulis F × *A. alpina* A	4/10/96	9	43 ♀	0	0	0	43	0
R. tenuicaulis F × *A. alpina* B	4/10/96	13	59 ♀	0	0	0	59	0
R. tenuicaulis E × *A. bellidioides* G/3	13/10/95	3	14 ♀	ND	0	9	5	0
R. tenuicaulis E × *A. bellidioides* G/5	11/10/95	8	40 ♀	ND	0	19	21	0
R. tenuicaulis C × *A. bellidioides* B	21/09/96	12	70 ♀	52	15	19	36	21
R. tenuicaulis E × *A. hookeri*	19/10/95	6	29 ♀	ND	0	9	20	0
R. tenuicaulis C × *A. trinervis* A	21/09/96	11	67 ♀	22	4	10	53	6
R. tenuicaulis C × *A. trinervis* B	29/09/96	6	35 ♀	ND	0	0	35	0
R. tenuicaulis B × *A. trinervis* B	23/09/96	9	52 ♀	8	0	0	52	0
R. tenuicaulis F × *A. trinervis* B	4/10/96	8	40 ♀	ND	0	0	40	0
R. tenuicaulis E × *H. lanceolatum* A	19/10/95	5	24 ♀	ND	0	6	18	0
R. tenuicaulis E × *P. luteoalbum* A	13/08/96	7	37 ♀	0	0	0	37	0
R. tenuicaulis D × *P. luteoalbum* B	13/08/96	4	26 ♀	0	0	0	26	0
R. tenuicaulis C × *R. haastii* A/1	19/09/96	2	12 ♀	7	2	2	8	17
R. tenuicaulis C × *R. haastii* A/4	19/09/96	4	23 ♀	9	1	5	17	4
A. bellidioides B × *R. tenuicaulis* C	22/09/96	3	all ♀	75 %	266	0	102	72
A. trinervis A × *R. tenuicaulis* C	19/09/96	5	all ♀	90 %	270	0	131	67
A. trinervis B × *R. tenuicaulis* B	23/09/96	4	all ♀	75 %	75	0	402	16
A. trinervis B × *R. tenuicaulis* F	1/10/96	3	all ♀	ND	15	0	619	2
E. audax B × *R. tenuicaulis* G	30/11/96	2	all ♀	90 %	120	3	121	49
E. delicatus × *R. tenuicaulis* C	16/09/96	1	all ♀	90 %	44	0	10	81
E. limosus C × *R. tenuicaulis* A	30/08/96	2	all ♀	ND	19	0	72	21
H. lanceolatum B/1 × *R. tenuicaulis* F	4/10/96	12	24 ♀	25 %	18	1	5	75
R. monroi C × *R. tenuicaulis* E	20/10/95	5	23 ♀	ND	2	0	21	9

Table 6.13. Experimental crosses with plants of *Raoulia tenuicaulis*. See See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ⚥, hermaphrodite florets.

Maternal Parent × Paternal Parent	Date	No. of capitula and florets pollinated		Style retraction	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		Capitula	florets					
A. alpina B × *H. intermedium* B	10/11/96	1	all ♀	0	0	4	127	0
A. alpina B × *R. haastii* A/1	22/09/96	2	all ♀	0	1	0	215	0.5
A. bellidioides I × *E. sinclairii* B	14/10/97	3	all ♀	ND	69	0	288	19
A. trinervis B × *E. sinclairii* C	31/10/96	1	all ♀	66%	4	1	167	2
E. audax A × *E. sinclairii* B	17/10/97	4	all ♀	75%	166	2	99	62
E. cf. *involucratus* × *L. grandiceps* B	16/11/96	3	all ♀	75 %	0	105	123	0
E. cf. *involucratus* × *R. hookeri* B	25/11/96	1	all ♀	ND	0	64	16	0
E. cf. *involucratus* × *R. monroi* A	25/11/96	1	all ♀	ND	0	0	91	0
E. limosus B × *H. filicaule* A	12/02/96	3	all ♀	ND	25	0	79	24
E. nitidulus B/1 × *R.* sp. "M"	13/11/96	1	all ♀	0	4	0	104	4
E. traversii B × *E. sinclairii* C	2/11/96	1	all ♀	10%	25	0	264	9
E. sinclairii B × *A. bellidioides* I	14/10/97	9	all ♀	90%	66	3	5	89
E. sinclairii A/3 × *O. leptophyllus* C	28/9/97	7	57 ♀, 18 ⚥	27	1	18	56	1
H. filicaule A × *E. limosus* B	12/02/96	1	31 ♀	0	0	0	31	0
H. filicaule B × *H. dimorphum*	25/01/96	10	all ♀	ND	1	7	192	0.5
H. filicaule A × *R. glabra* A	10/03/96	7	all ♀	ND	1	1	84	1
H. intermedium B × *E. sinclairii* C	11/11/96	1	18 ♀	ND	1	0	17	6
H. parvifolium × *R. subsericea* A	21/12/96	1	7 ♀	2	4	0	3	57
P. luteoalbum D × *A. trinervis* D	24/11/96	2	all ♀	0	1	0	179	0.6
R. apicinigra C × *L. grandiceps* B	27/11/96	3	8 ♀, 25 ⚥	0	0	0	33	0
R. apicinigra B × *R.* sp. "M"	20/11/96	3	14 ♀, 12 ⚥	0	0	0	26	0
R. beauverdii A × *R.* sp. "M"	15/11/96	1	8 ♀, 8 ⚥	0	0	0	16	0
R. mammillaris A/1 × *R. albosericea* B	21/12/97	4	all ♀	ND	21	0	7	75
R. mammillaris A/1 × *R. grandiflora* C/5	14/12/97	5	all ♀	90 %	18	7	9	53
R. mammillaris A/1 × *R. subsericea* B	13/12/97	8	all ♀	100%	43	1	7	84
R. monroi C/3 × *R. haastii* A/1	27/09/96	5	27 ♀, 1 ⚥	0	0	0	28	0
R. sp. "M" × *L. grandiceps* B	17/11/96	1	12 ♀, 1 ⚥	0	0	0	13	0
R. sp. "M" × *R. grandiflora* C/1	20/11/96	1	7 ♀, 2 ⚥	0	0	0	9	0
R. subsericea B × *R. grandiflora* C/5	12/12/97	2	all ♀	0	0	0	46	0

Table 6.14. Experimental crosses between other indigenous Gnaphalieae. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ⚥, hermaphrodite florets.

6.3.4 Experimental crosses between indigenous and exotic Gnaphalieae

Anaphalioides trinervis

Seven crosses with plants of exotic Gnaphalieae were performed (Table 6.15 p. 293). Germination of *Antennaria dioica*, *Leontopodium palibinianum* and *Vellereophyton dealbatum* pollen grains on *A. trinervis* stigmas was observed at a low estimated frequency. Of all germinated grains observed, 98 % were attached to the stigma. No germinated *Gamochaeta spicata* pollen grains were observed on the stigma of *A. trinervis*. Style retraction following pollination was not observed in any of the crosses. In a single cross with a plant of *Ewartia planchonii*, 6 % of the cypselas were enlarged but empty, and 5 % of the cypselas were filled in a single cross with *V. dealbatum*.

Euchiton audax

Twelve crosses with plants of exotic Gnaphalieae were performed (Table 6.16 p. 294). Germination of *Antennaria dioica*, *Gamochaeta spicata*, *Leontopodium palibinianum* and *Vellereophyton dealbatum* pollen grains was observed on *E. audax* stigmas at a low estimated frequency. Pollen gains of *E. audax* germinated at a low frequency on the stigma of plants of *Anaphalis margaritacea* and *G. spicata*, but no germinated grains were observed on the stigma of a plant of *V. dealbatum*. Of all germinated grains observed, 95 % were attached to the stigma. Style retraction occurred at a high frequency in all eight crosses in which a plant of *E. audax* was the maternal parent, but was not observed in three crosses in which *E. audax* was the paternal parent. The styles retracted within 1.5–2 h after pollination in all crosses in which *E. audax* was the maternal parent. In these crosses pollen grains had germinated within 1 h after pollination, before the styles had visibly begun to retract. Filled cypselas were obtained in both directions of a single reciprocal cross between plants of *E. audax* and *Gamochaeta spicata*; seeds were more frequent when *E. audax* was the maternal parent. Plants of *E. audax* and *Ewartia planchonii* possessed low cross-compatibility (based on a single cross). Enlarged but empty cypselas were produced in all crosses in which a plant of *E. audax* was the maternal parent, but not when *E. audax* was the paternal parent.

Pseudognaphalium luteoalbum

Nine crosses with plants of exotic Gnaphalieae were performed (Table 6.17 p. 294). Germination of *Antennaria dioica*, *Gamochaeta spicata*, *Leontopodium palibinianum* and *Vellereophyton dealbatum* pollen grains was observed on *P. luteoalbum* stigmas at a low estimated frequency. Germinated pollen grains of *P. luteoalbum* were observed at a low frequency on *Anaphalis margaritacea*, *G. spicata* and *V. dealbatum* stigmas. A high frequency of style retraction in *P.*

luteoalbum florets occurred within 1.5 h after pollination by plants of *Ant. dioica* and *V. dealbatum* (based on single crosses). Less than 5 % of the cypselas were filled in single crosses with *Ant. dioica*, *G. spicata* and *V. dealbatum*. Enlarged, empty cypselas were absent or rare in most crosses, but comprised 87 % of the cypselas in the cross *P. luteoalbum* C × *V. dealbatum*.

Other indigenous Gnaphalieae

Seventeen crosses between other indigenous and exotic Gnaphalieae were performed (Table 6.18 p. 295). Germination of *Ozothamnus leptophyllus* pollen was observed at low or moderate frequency on *Gamochaeta spicata* and *Vellereophyton dealbatum* stigmas, but pollen germination was not investigated in the other crosses. The lowest number of germinated *O. leptophyllus* pollen grains observed was on *V. dealbatum* stigmas. Style retraction after pollination was only observed in crosses employing a *Euchiton* species as the maternal parent. Filled cypselas were not obtained in any cross. Enlarged but empty cypselas were produced in the six crosses in which a *Euchiton* species was the female parent (at a frequency of 20–92 %) and in a single cross between plants of *Ewartia planchonii* and *O. leptophyllus* (in 6 % of the pollinated florets).

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Estimate of pollengermination (%)	Style retraction after pollination (%)	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set
		Capitula	Florets						
A. trinervis D × *A. dioica*	21/11/96	2	all ♀	33	0	0	0	414	0
A. trinervis D × *G. spicata*	26/11/97	2	all ♀	0	0	0	0	c.300	0
A. trinervis D × *L. palibinianum*	9/12/96	3	all ♀	1	0	0	0	274	0
A. trinervis D × *L.* sp.	21/11/96	1	all ♀	ND	0	0	0	289	0
A. trinervis D × *V. dealbatum*	26/11/97	2	all ♀	19	0	0	0	c.300	0
E. planchonii C/1 × *A. trinervis* D	26/9/97	2	all ♀	ND	0	0	5	81	0
V. dealbatum × *A. trinervis* D	26/11/97	4	all ♀	ND	0	4	0	83	5

Table 6.15. Results of experimental crosses between plants of *Anaphalioides trinervis* and exotic Gnaphalieae. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Estimate of pollengermination (%)	Style retraction after pollination (%)	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set
		Capitula	Florets						
E. audax B × *A. dioica*	17/11/96	6	all ♀	29	75	0	385	77	0
E. audax D × *A. dioica*	27/11/96	2	all ♀	ND	90	0	96	40	0
E. audax B × *G. spicata*	25/11/97	6	all ♀	3	> 90	302	138	28	65
E. audax B × *L. palibinianum*	26/12/96	6	all ♀	9	90	0	231	153	0
E. audax B × *L.* sp.	24/11/96	3	all ♀	ND	66	0	59	130	0
E. audax B × *O. hookeri*	24/12/96	1	all ♀	ND	90	0	43	11	0
E. audax A × *O.* sp.	30/10/97	6	all ♀	ND	90	0	335	111	0
E. audax B × *V. dealbatum*	1/12/96	2	all ♀	12	75-90	0	47	101	0
A. margaritacea × *E. audax* B	6/1/98	2	all ♀	5	0	0	0	c. 300	0
E. planchonii C/1 × *E. audax* A	20/10/96	1	all ♀	ND	ND	5	0	27	16
G. spicata × *E. audax* B	26/11/97	3	all ♀	8	0	22	0	94	19
V. dealbatum × *E. audax* B	2/1/98	5	all ♀	0	0	0	0	c.90	0

Table 6.16. Results of experimental crosses between plants of *Euchiton audax* and exotic Gnaphalieae. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Estimate of pollengermination (%)	Style retraction after pollination (%)	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set
		Capitula	Florets						
P. luteoalbum C × *A. dioica*	20/1/98	4	all ♀	19	> 90	0	21	c.280	0
P. luteoalbum D × *A. dioica*	24/11/96	1	all ♀	ND	0	2	1	65	3
P. luteoalbum C × *G. spicata*	6/12/97	3	all ♀	5	0	7	6	251	3
P. luteoalbum C × *L. palibinianum*	26/11/97	4	all ♀	6	0	0	0	c.450	0
P. luteoalbum D × *L.* sp.	24/11/96	2	all ♀	ND	0	0	0	246	0
P. luteoalbum C × *V. dealbatum*	30/12/97	4	all ♀	16	> 90	0	285	43	0
A. margaritacea × *P. luteoalbum* C	4/1/98	3	all ♀	6	0	0	0	c. 450	0
G. spicata × *P. luteoalbum* C	26/11/97	6	all ♀	21	0	5	6	358	1
V. dealbatum × *P. luteoalbum* C	1/1/98	5	all ♀	0.3	0	1	0	c.90	1

Table 6.17. Results of experimental crosses between plants of *Pseudognaphalium luteoalbum* and exotic Gnaphalieae. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Estimate of pollen germination (%)	Style retraction after pollination (%)	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set
		Capitula	Florets						
***A. bellidioides* C** × *A. dioica*	24/11/9	1	all ♀	ND	0	0	0	181	0
E. delicatus × *A. dioica*	25/11/9	2	all ♀	ND	90 %	0	133	12	0
E. delicatus × *L.* sp.	27/11/9	1	all ♀	ND	ND	0	16	49	0
E.* cf. *involucratus × *A. dioica*	17/11/9	5	all ♀	ND	90 %	0	280	85	0
E.* cf. *involucratus × *L. palibinianum*	3/12/96	2	all ♀	ND	100	0	79	39	0
E.* cf. *involucratus × *L.* sp.	16/11/9	4	all ♀	ND	75 %	0	116	135	0
***E. traversii* B** × *L.* sp.	3/11/96	1	all ♀	ND	ND	0	64	250	0
E. meredithae × ***E. nitidulus* B**	30/10/9	2	25 F	ND	0	0	0	25	0
E. meredithae × ***E. sinclairii* C**	20/10/9	2	26 F	ND	0	0	0	26	0
E. meredithae × ***L. leontopodium* A**	20/10/9	2	32 F	ND	0	0	0	32	0
E. meredithae × ***R. grandiflora* A**	20/10/9	1	13 F	ND	0	0	0	13	0
E. planchonii C/1 × ***O. leptophyllus* C**	26/9/97	2	all ♀	ND	0	0	5	74	0
G. spicata × ***O. leptophyllus* D**	6/12/97	6	all ♀	36	0	0	0	313	0
***L. grandiceps* A** × *L.* sp.	27/11/9	3	29 ♀,26	ND	0	0	0	55	0
***L. leontopodium* A** × *L.* sp.	3/11/96	3	all ♀	ND	0	0	0	c. 80	0
***L. leontopodium* B** × *L.* sp.	14/11/9	10	all ♀	ND	0	0	0	c. 200	0
V. dealbatum × ***O. leptophyllus* D**	5/12/97	9	all ♀	10	0	0	0	141	0

Table 6.18. Results of experimental crosses between other indigenous and exotic Gnaphalieae. The names of indigenous species are in bold. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ⚥, hermaphrodite florets.

6.3.5 Experimental crosses among exotic Gnaphalieae

Ten crosses between single plants of five exotic Gnaphalieae were performed (Table 6.19 p. 296). In all crosses in which pollen germination on the stigma was investigated except *Vellereophyton dealbatum* × *Gamochaeta spicata*, germinated grains were observed at a low estimated frequency. Of all germinated grains observed, 94 % were attached to the stigma. Style retraction (within 2 h after pollination) occurred only in the cross *Anaphalis margaritacea* × *V. dealbatum*. Plants of *Antennaria dioica* and *Ewartia planchonii* had a moderate cross-compatibility (based on a single cross). In separate crosses between the plant of *G. spicata* and the *Ant. dioica* and *V. dealbatum* plants, 1–2 % of the cypselas were filled. Enlarged, empty cypselas were produced only in single crosses between *Ant. dioica* and *E. planchonii*, and between *G. spicata* and *V. dealbatum*.

Maternal parent × Paternal parent	Date crossed	No. of capitula and florets pollinated		Estimate of pollen germination (%)	Style retraction after pollination (%)	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set
		Capitula	Florets						
A. margaritacea × *A. dioica*	19/1/98	6	all ♀	15	0	0	0	c. 300	0
A. margaritacea × *G. spicata*	23/1/98	1	all ♀	4	0	0	0	c. 150	0
A. margaritacea × *L. palibinianum*	4/1/98	2	all ♀	15	0	0	0	c. 300	0
A. margaritacea × *V. dealbatum*	5/1/98	2	all ♀	35	75	0	0	c. 300	0
E. meredithae × *E. planchonii* C/2	20/10/9	1	20 F	ND	0	0	0	20	0
E. planchonii C/3 × *A. dioica*	15/9/97	3	all ♀	ND	90 %	37	28	36	37
G. spicata × *A. dioica*	19/1/98	2	all ♀	15	0	1	7	107	1
G. spicata × *L. palibinianum*	26/11/9	3	all ♀	21	0	0	0	c.180	0
G. spicata × *V. dealbatum*	25/11/9	4	all ♀	13	0	0	32	187	0
V. dealbatum × *A. dioica*	23/1/98	6	all ♀	4	0	0	0	c. 110	0
V. dealbatum × *G. spicata*	23/11/9	6	all ♀	0	0	2	0	98	2
V. dealbatum × *L. palibinianum*	1/1/98	6	all ♀	2	0	0	0	c.110	0

Table 6.19. Pollen germination and seed set in crosses between exotic Gnaphalieae. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded.

6.3.6 Experimental crosses involving natural putative Gnaphalieae hybrids

Six crosses were performed between a natural *Anaphalioides bellidioides* × *Helichrysum lanceolatum* hybrid and plants from the putative parental species, all of which yielded filled cypselas (Table 6.20 p. 297). However, seed set was considerably higher when an *A. bellidioides* plant was the maternal parent than when *H. lanceolatum* was the pollen recepient. In addition, seed set was higher when *A. bellidioides* was the pollen donor than when *H. lanceolatum* was the paternal parent.

Filled cypselas were produced in crosses between *A. bellidioides* and the cultivated hybrid 'Graeme Paterson', and between *H. lanceolatum* and a putative *H. intermedium* × *H. lanceolatum* hybrid. However, a putative *H. dimorphum* × *H. filicaule* hybrid failed to set any seed when pollinated by a plant of *H. filicaule.*

Maternal Parent × Paternal Parent	Date	No. of capitula and florets pollinated		Style retraction	Filled enlarged cypselas	Empty enlarged cypselas	Unenlarged cypselas	Seed set (%)
		Capitula	florets					
A. bellidioides B × (*A. bellidioides* × *H. lanceolatum*) A	30/09/96	4	all ♀	ND	281	0	177	61
A. bellidioides F × (*A. bellidioides* × *H. lanceolatum*) A	7/10/96	2	all ♀	ND	211	0	76	74
(*A. bellidioides* × *H. lanceolatum*) A × *A. bellidioides* B	28/09/96	8	all ♀	90 %	25	5	146	14
(*A. bellidioides* × *H. lanceolatum*) A × *A. bellidioides* F	5/10/96	7	all ♀	75 %	32	0	77	29
(*A. bellidioides* × *H. lanceolatum*) A × *H. lanceolatum* B/2	9/10/96	6	all ♀	ND	1	0	98	1
H. lanceolatum B/2 × (*A. bellidioides* × *H. lanceolatum*) A	26/10/96	3	7 ♀, 22 ⚥	ND	1	0	28	3
A. bellidioides H × 'Graeme Paterson'	27/10/96	2	all ♀	90 %	86	0	181	32
'Graeme Paterson' × *H. intermedium* var. *tumidum*	7/11/96	3	all ♀	90 %	0	69	86	0
A. bellidioides I/3 × 'Graeme Paterson'	2/11/97	2	all ♀	0	0	0	209	0
(*H. dimorphum* × *H. filicaule*) A/2 × *H. filicaule* C	10/1/96	12	all ♀	0	0	0	142	0
H. lanceolatum C/1 × (*H. intermedium* × *H. lanceolatum*) A/2	26/10/97	6	6 ♀, 32 ⚥	ND	27	5	16	56
R. mammillaris A/1 × (*R. hectorii* × *R. subsericea*) B	20/12/97	5	all ♀	0	2	0	30	6

Table 6.20. Experimental crosses with natural putative hybrids. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances. ND, data not recorded. ♀, female florets; ⚥, hermaphrodite florets.

6.3.7 Pollen stainability of experimental and putative natural intergeneric hybrids

Artificial hybrids

Pollen from 24 artificial intergeneric hybrids, raised from ten different experimental crosses, was stained (Table 6.21 p. 299). In only one hybrid – from an *Euchiton* cf. *involucratus* × *Helichrysum intermedium* cross (Plate 14 A p. 300) – were all pollen grains abnormal. In this hybrid, no pollen was presented on the unopened stigma arms and pollen grains had to be dissected from the anthers. Pollen stainability was also low in an *Euchiton limosus* × *Raoulia tenuicaulis* hybrid (Plate 14 B) and ten *E. limosus* × *H. filicaule* hybrids. The frequency of normal pollen grains in the other hybrids varied widely. Over 90 % of the pollen grains were normally developed in a *Raoulia tenuicaulis* × *Anaphalioides trinervis* hybrid. In contrast, less than 5 % of the pollen grains were normal in each of ten *E. limosus* × *H. filicaule* hybrids. Among three *A. trinervis* × *Leucogenes leontopodium* hybrids, pollen stainability ranged from 24 % to 67 %. Pollen stainability was low to moderate in two *A. bellidioides* × *R. tenuicaulis* hybrids (Plate 14 C) and an *A. bellidioides* × *R. monroi* hybrid (Plate 14 D). In three backcross

hybrids between *A. bellidioides* and a natural *A. bellidioides* × *H. lanceolatum* hybrid, over 70 % of the pollen grains were normal. This proportion was higher than in the natural hybrid (see Table 6.22 p. 301). Pollen stainability in a fourth backcross hybrid and an artificial F_1 *A. bellidioides* × *H. lanceolatum* hybrid was similar to that of the natural hybrid.

Natural putative hybrids

The pollen stainability of seven putative intergeneric hybrids varied widely (Table 6.22 p. 301). Pollen stainability exceeded 80 % in the cultivar 'Graeme Paterson', a natural hybrid between *H. intermedium* var. *tumidum* and *A. bellidioides*. In contrast, a putative *A. bellidioides* × *L. grandiceps* hybrid from Mt Hutt produced no normal pollen grains. Two putative *H. dimorphum* × *H. filicaule* hybrids possessed low pollen stainability. In these plants some of the peripheral filiform florets bore one to three anthers, but pollen was not presented on the unopened stigma arms and less than 5 % of the grains were normal. The proportion of normal pollen grains in the central hermaphrodite florets was only slightly higher.

Pollen stainability in the putative intrageneric hybrids was also variable. Pollen stainability exceeded 90 % in a putative *H. intermedium* × *H. lanceolatum* hybrid, an *A. bellidioides* × *A. trinervis* hybrid and four putative hybrids among members of *Raoulia* subg. *Raoulia* (*R. apicinigra* × *R. australis*, *R. australis* × *R. beauverdii*, *R. australis* × *R. parkii* and *R. australis* × *R. subsericea*). In contrast, the proportion of normal pollen grains was less than 20 % in a putative *R. apicinigra* × *R. cinerea* hybrid. Putative *H. coralloides* × *H. depressum* and *R. hectorii* × *R. subsericea* hybrids possessed an intermediate level of normal pollen grains.

6.4 Discussion

Although compatible crosses provide evidence for relationships, the absence of seed set is not necessarily proof of the incompatibility of two plants. A variety of internal reproductive barriers can cause cross-incompatibility (see Grant, 1981 pp. 111–117), so results need to be interpreted with caution. Reproductive barriers can exist between populations of a species or within a population (Ornduff, 1964; Ornduff, 1966). As Stuessy (1990 p. 326) emphasised, crossing programs need to be as complete as possible to allow conclusions to be drawn and the results interpreted with confidence. A complete programme of reciprocal crosses was not possible in this thesis. The crosses performed were dictated by flower availability, whether flowering periods overlapped and numerous practical obstacles that had to be overcome. The flowering periods vary widely between species and individual plants may be in flower for less

Hybrid	No. of counts	Normal grains	Abnormal grains	Mean normal (%) ± s.d.
A. bellidioides B × *(A. bellidioides* × *H. lanceolatum)* A	6	961	272	77.9 ± 4.5
A. bellidioides B × *(A. bellidioides* × *H. lanceolatum)* A	5	546	475	53.4 ± 7.2
(*A. bellidioides* × *H. lanceolatum*) A × *A. bellidioides* B	5	735	284	72.0 ± 8.7
(*A. bellidioides* × *H. lanceolatum*) A × *A. bellidioides* B	4	646	161	80.1 ± 3.7
A. bellidioides B × *R. monroi* C/3	6	479	721	39.9 ± 2.3
A. bellidioides B × *R. tenuicaulis* C	6	385	904	29.9 ± 3.6
A. bellidioides B × *R. tenuicaulis* C	6	259	990	20.7 ± 3.1
A. trinervis D × *L. leontopodium* A	6	798	402	66.5 ± 7.1
A. trinervis D × *L. leontopodium* A	6	532	668	44.1 ± 7.5
A. trinervis D × *L. leontopodium* A	6	291	909	24.3 ± 6.0
E. cf. *involucratus* × *H. intermedium* B	6	0	1200	0
E. limosus B × *H. filicaule* B	3	14	586	2.3 ± 0.8
E. limosus B × *H. filicaule* B	3	14	639	2.2 ± 1.1
E. limosus B × *H. filicaule* B	3	19	581	3.2 ± 1.6
E. limosus B × *H. filicaule* B	3	7	598	1.2 ± 0.6
E. limosus B × *H. filicaule* B	3	23	577	3.8 ± 1.3
E. limosus B × *H. filicaule* B	3	11	590	1.8 ± 1.2
E. limosus B × *H. filicaule* B	3	6	601	1.0 ± 0.0
E. limosus B × *H. filicaule* B	3	6	614	0.9 ± 0.7
E. limosus B × *H. filicaule* B	3	19	582	3.2 ± 0.6
E. limosus B × *H. filicaule* B	3	14	586	2.3 ± 1.2
E. limosus B × *R. tenuicaulis* C	6	274	926	22.8 ± 8.7
H. lanceolatum B/2 × *A. bellidioides* F	6	745	455	62.1 ± 5.1
R. tenuicaulis C × *A. trinervis* A	6	1115	85	92.9 ± 2.0

Table 6.21. Pollen stainability of experimental intergeneric hybrids among New Zealand Gnaphalieae. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances.

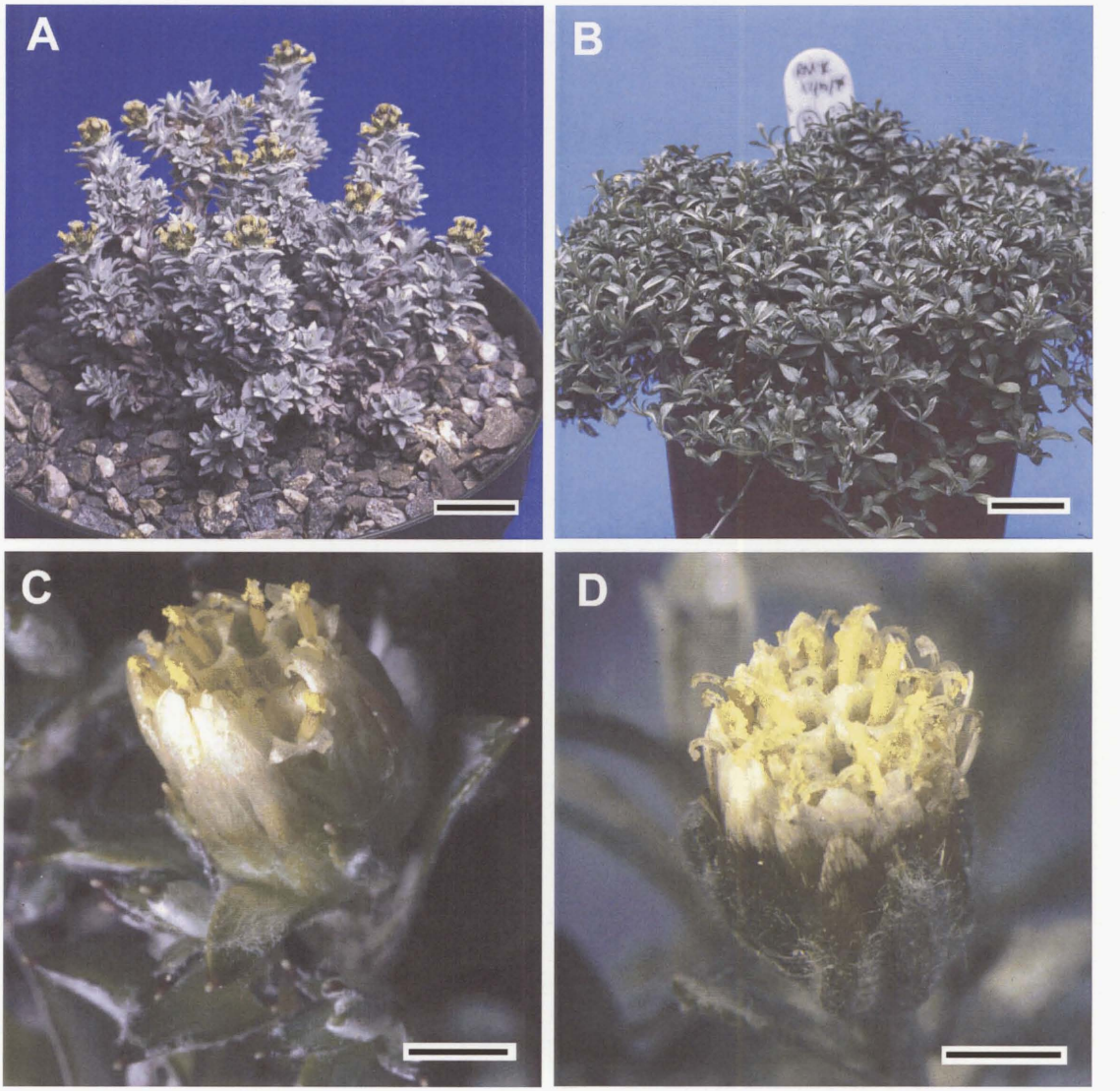
Plate 14. Experimental Gnaphalieae hybrids synthesised during this thesis.

A, *Euchiton* cf. *involucratus* × *Helichrysum intermedium* B. Scale bar = 2 cm. (photo Dougal Holmes)

B, *Euchiton limosus* B × *Raoulia tenuicaulis* C. Scale bar = 2 cm. (photo Dougal Holmes)

C, *Anaphalioides bellidioides* B × *Raoulia tenuicaulis* C. Scale bar = 2 mm.

D, *Anaphalioides bellidioides* B × *Raoulia monroi* D. Scale bar = 2 mm.


A
B
C
D

Wild putative hybrid	No. of counts	Normal grains	Abnormal grains	Mean normal (%) ± s.d.
A. bellidioides × *H. lanceolatum* A	6	654	554	54.5 ± 6.8
A. bellidioides × *H. lanceolatum* B	6	880	320	73.3 ± 9.5
A. bellidioides × *A. trinervis*	6	1152	48	96.0 ± 3.6
A. bellidioides × *L. grandiceps*	6	0	1200	0
H. coralloides × *H. depressum*	6	624	595	51.1 ± 4.5
H. dimorphum × *H. filicaule* A/1				
central ♀florets	3	76	539	12.3 ± 2.2
peripheral ♀florets	6	53	1147	4.4 ± 1.2
H. dimorphum × *H. filicaule* A/2				
central ♀florets	6	103	1097	8.6 ± 2.4
peripheral ♀florets	6	33	1196	2.7 ± 1.0
H. filicaule × *R. glabra*	6	489	711	40.8 ± 2.2
H. intermedium × *H. lanceolatum* A/2	6	1145	55	95.4 ± 3.3
(*H. intermedium* var. *tumidum* × *A. bellidioides*) 'Graeme Paterson'	6	1027	195	84.1 ± 2.0
R. apicinigra × *R. australis*	6	1169	33	97.3 ± 3.3
R. apicinigra × *R. cinerea*	3	106	494	17.7 ± 1.9
R. australis × *R. beauverdii*	6	1182	58	94.3 ± 4.5
R. australis × *R. parkii*	6	1178	22	98.2 ± 1.7
R. australis × *R. subsericea*	3	598	2	99.7 ± 0.3
R. hectorii × *R. subsericea* A	3	329	283	53.7 ± 2.8
R. hectorii × *R. subsericea* B	6	797	408	66.4 ± 6.2

Table 6.22. Pollen stainability of natural putative hybrids among the New Zealand Gnaphalieae. Putative hybrids studied in Chapters 4 and 5 are not included. See Table 6.2 (p. 269) for the full generic names and Appendix 2 for plant provenances.

than four weeks (Wilton, 1997). Compositae pollen has very short viability (Brewbaker, 1967), at least under humid conditions (Hoekstra and Bruinsma, 1975). The actual pollination process was technically demanding and very time consuming, as the florets are tiny, difficult to manipulate and tightly congested within the capitula. The New Zealand Gnaphalieae comprise a large group of species (about 70–80 spp.) and artificial crossing of species across the entire group is a long-term project, so 'target' species were selected based on their availability and ease of cultivation in the glasshouse.

A variety of internal and external factors can influence cross-compatibility. Maternal choice, such as selective abortion of seeds retaining only those of "high genetic quality", might occur when resources are limited (Sutherland, 1986). In *Aster furcatus* resource allocation was suggested to be positively correlated with floret number per capitulum following self-pollination (Reinartz and Les, 1994), but in *Centaurea scabiosa* L. fruit set in individual capitula was

unaffected by the total number of capitula produced (Ehlers, 1999). Given the low number of florets pollinated per plant and the use of cultivated plants in the present study, resource limitations are unlikely to have been a major influence on seed set. The self-incompatibility (SI) system can obstruct outcrossing. In Compositae, which possess sporophytic SI, different genotypes will be incompatible if they share one *S* allele (Richards, 1997 p. 226). Variation in the stage at which cross-incompatibility was expressed, both within and between crosses, suggests a number of internal factors might contribute to cross-incompatibility in the New Zealand Gnaphalieae. For example, in the cross *Raoulia tenuicaulis* C × *Anaphalioides bellidioides* B the style retracted in 63 % of the florets, the cypsela enlarged in 49 % and seeds matured in only 21 % of the florets.

Performance of all crosses under a stereo microscope helped to ensure consistency in the pollen load applied and ensured no stigmas precontaminated with self- or cross-pollen were pollinated. However, some variation in the pollen load is inevitable and not all pollen grains would have touched the stigmatic surface and had the opportunity of germinating. Contamination via insects or air movement within the exclosures is unlikely to have been a major factor in compatible crosses; only rarely were filled cypselas obtained from emasculated, unpollinated capitula. In all instances where seeds from a cross were sown, the hybrid origin of all seedlings and the absence of 'rogue' seedlings was confirmed. Plants were periodically treated with an insecticide and miticide, but the possibility of pollen predation by mites and thrips cannot be discounted. Removal of hermaphrodite florets prior to pollen presentation, almost exclusive pollination of female florets, and controlled environmental conditions ensured that mentor effects (see de Nettancourt, 1977 p.70-71) did not affect the results. Only freshly presented pollen was used, ensuring loss of pollen viability was not a factor in the results. Pollen quality was not tested prior to each cross and thus variation in pollen quality cannot be discounted as a contributing factor to variation in cross-compatibility. Lloyd (1965) found that cross-compatibility results can differ from year to year. All plants were grown and pollinations performed under the same conditions, but variation in plant vigour and environmental conditions from the adaptive optimum for each species cannot be excluded as contributing factors. There was no indication any of the Gnaphalieae plants used were agamospermous, at least under the environmental conditions used.

A number of internal pre- and post-zygotic barriers were indicated to be important in incompatible interspecific crosses: failure of pollen germination on the stigma (e.g., *A. trinervis* × *Euchiton audax*); failure of cypsela enlargement despite pollen germination (e.g., *A. trinervis* ×

Ozothamnus leptophyllus); and development of empty cypselas (e.g., crosses using *Euchiton* plants as the maternal parent). Further investigations into the point of incompatibility would be informative, and also whether style retraction and the production of enlarged, empty cypselas are pre-zygotic or post-zygotic responses to pollination. Style retraction might be a response to pollen-tube growth in the style rather than fertilisation, at least in *Euchiton* species, in which the response is rapid (within 2 h of pollination). In some crosses (e.g., *Raoulia tenuicaulis* C × *Anaphalioides bellidioides* A and *E. audax* B × *R. bryoides* B), the number of enlarged cypselas was notably less than the number of retracted styles, suggesting style retraction is a pre-zygotic response or early zygotic abortion occurred before some cypselas had visibly enlarged. In most crosses where such data was collected, estimates of the proportion of retracted styles was consistent with or exceeded the proportion of mature cypselas or enlarged, unfilled cypselas produced.

Self-fertility and autonomous selfing

Seed set following self-pollination suggested the individual plants tested were either highly self-incompatible or strongly self-compatible, but further experimentation is required to determine the constancy of the breeding system at the individual, population and species levels. Variation in the degree of self-compatibility within populations or taxa have been reported in other Compositae (Andersson, 1989; Reinartz and Les, 1994; Byers, 1995; Hiscock, 2000a; Young *et al.*, 2000). Hiscock (2000b) concluded the normally self-incompatible *Senecio squalidus* has a flexible breeding system allowing a certain level of self-compatibility. High temperatures, high concentrations of gaseous CO_2, electrical stimuli and damage to the stigmatic cuticle can overcome SI in *Brassica* and *Primula* (Richards, 1997 pp. 229-230). Thus, a range of environmental variables might have precluded self-fertilisation in plants implicated to be self-incompatible in the present study.

The high level of autonomous selfing in *Euchiton* plants studied indicates the capitula are well adapted for self-fertilisation. Energy expenditure on pollen and adaptations that facilitate outcrossing are predicted to decline with increased frequency of autogamous self-fertilisation (Lloyd, 1987). The capitulum structure, pollen:ovule ratio and flowering phenology of *E. audax* and *E. traversii* (Wilton, 1997) and the level of autonomous selfing are consistent with a reduced investment in pollen associated with adaptations to increase the frequency of vector-independent geitonogamous self-fertilisation. As observed by Wilton (1997), the style arms of the female florets are available to receive outcross pollen before self-pollen is presented and *E. audax*

produces nectar. Vector-independent self-fertilisation provides reproductive assurance in conditions when vector-mediated pollination is insufficient for full seed set (Schoen and Lloyd, 1992). Wilton (1997) did not observe any floral visitors to *E. audax* and *E. traversii*, suggesting a paucity of pollinator visits, at least at the site monitored, but air currents and vibration of the flowering shoots in the field might promote selfing by transferring pollen within and between capitula in the congested inflorescences. Owing to the sticky nature of the pollen, wind-mediated cross-pollination seems unlikely.

Self-fertility might be an adaptation to reduce energetic costs in an energy-limited habitat, as suggested by Lloyd (1965) for self-compatible races in *Leavenworthia.* A breeding system that tends to minimise genetic variability (such as self-fertilisation) will be favoured in extreme habitats, which impose severe stabilising selection (Richards, 1997 pp. 453–454). In *Espeletia* self-compatibility (in association with adaptations for wind pollination) has been interpreted as a response to lower pollinator availability at higher altitudes (Berry and Calvo, 1989). Self-compatibility has also been associated with an annual to biennial, weedy, colonising habit and establishment after long-distance dispersal (Baker, 1974; Pandey, 1979). *Euchiton audax* and *E. traversii* have been termed 'opportunists' (Wilton, 1997) and commonly grow in open, sparsely vegetated or disturbed sites, such as in grassland, on roadsides and on riverbeds, but some *Euchiton* species prefer wet, densely vegetated situations such as swamps and bogs (Drury, 1972). Self-fertility is advantageous for an opportunistic life style, allowing populations to establish from single propagules.

Differences in capitulum structure would account for the different levels of autonomous selfing in self-compatible *Anaphalioides* and *Euchiton* plants. In *E. audax* and *E. traversii*, the florets are tightly congested within the capitulum and pollen is presented at a similar level to the style arms of the female florets. In *A. bellidioides* the female and hermaphrodite florets are spatially better separated and pollen is presented well above the style arms of the female florets (Wilton, 1997). The capitulum structure of *A. alpina, A. hookeri* and *A. trinervis* is very similar to *A. bellidioides*. The lower level of autonomous selfing in self-compatible *Anaphalioides* plants (0.5–56 %) than in *Euchiton* plants (62–94 %) indicates a vector is more important for geitonogamy in self-compatible *Anaphalioides*. Other Compositae with high levels of autonomous selfing possess adaptations considered to be associated with inbreeding, such as smaller, less showy capitula, a lower pollen:ovule ratio, a different corolla colour from related outcrossers or less distinct protandry in central florets of the capitulum (Sun and Ganders, 1988; Andersson, 1989; Mejias, 1992; Mejias, 1994; Nicholls, 2000). The frequency of selfing in the

field will also be influenced by factors such as environmental conditions and 'prepotency' (the success of cross-pollen in achieving fertilisation in competition with self-pollen) (Lloyd and Schoen, 1992).

Non-reciprocity of cross-compatibility

Non-reciprocity of crosses appears to be common in the Compositae (e.g., Ornduff, 1964; Ornduff, 1966; Christov and Panayotov, 1991; Abbott and Lowe, 1996; Young *et al.*, 2000). In this thesis, non-reciprocity occurred in crosses between two SI plants (e.g., *Anaphalioides bellidioides* and *Helichrysum intermedium*), two SC plants (e.g., *A. trinervis* and *Euchiton audax*) and between SC and SI plants (e.g., *Euchiton audax* and *H. intermedium*). Thus, unilateral incompatibility (as defined by Lewis and Crowe, 1958) is not a feature of the New Zealand Gnaphalieae. Variation in the post-pollination stage at which cross-incompatibility was expressed occurred both within and between crosses, suggesting a number of internal factors contribute to non-reciprocity of interspecific crosses in the Gnaphalieae. In some crosses cross-compatibility between two species varied depending on the provenance of the parents (e.g., crosses between *A. bellidioides* and *H. intermedium*). Differences in the behaviour of *Euchiton* and self-compatible *Anaphalioides* plants in experimental crosses suggests different underlying genetic controls of self-compatibility in the two genera. Various mutations can give rise to SC (de Nettancourt, 1977 pp. 112–133). When *Euchiton* plants were the maternal parent, cross-compatibility appeared to be limited only by genetic disharmony (e.g., taxonomic distance or differing ploidy levels), but cross-compatibility of *A. trinervis* was less predictable in experimental crosses. A mutation preventing expression of the SI reaction in *Euchiton* pistils might, in the absence of any other mate-selection mechanisms, allow fertilisation by any genetically compatible plant. Differences in cross-reciprocity among self-compatible plants in this thesis also suggests maternal choice of mates in SI plants, and its absence in self-compatible plants, is unlikely to be a characteristic in the plants used.

Various explanations for non-reciprocity of crosses have been proposed. In Compositae, which are considered to possess sporophytic SI, non-reciprocal compatibility between different genotypes will occur when the two parents share one SI allele ('*S* allele') (Richards, 1997 p. 226). Dominance, interaction or mutual weakening of the *S* alleles in the anther and stigma could also be a factor. In *Rutidosis leptorrhynchoides* F.Muell. one-way compatibility and reciprocal incompatibility is more likely with increasing relatedness of the parents (Young *et al.*, 2000). A number of hypotheses have sought to explain cross reciprocity differences between SC and SI plants in other families. Lewis and Crowe (1958) suggested the *S* locus had a dual function in

both self- and interspecific incompatibility in gametophytic and sporophytic systems. Strongest support for UI came from crosses in the Solanaceae, a family possessing a gametophytic SI system. Subsequent studies have demonstrated differences between SI and UI in *Lycopersicon* (Liedl *et al.*, 1996) and loci other than the *S* locus are implicated in UI in the Cruciferae (Lewis *et al.*, 1988). Hogenboom (1975) suggested interspecific incompatibility is caused by genic disharmony between the pistil and pollen grain and is unrelated to the SI system. Arnold and Richards (1998) suggested cytoplasmic-nuclear DNA interactions or an embryo-endosperm imbalance could explain reciprocal differences in cross-compatibility between SC and SI *Primula* L. species, which possess a heteromorphic sporophytic SI system. De Nettancourt (1997) suggested self-compatible pollen lacks the specific *S* information necessary to either block access to the pollen tube of *S*-RNases from the SI stigma, or to inactivate the *S*-RNases.

To explain non-reciprocity in the present study, identification of the locus or loci responsible for cross reciprocity is essential. If the *S* locus has a role, knowledge of the *S* genotype of each plant and determination of the dominance hierarchy and interactions between *S* alleles must be determined. Trans-specific evolution of *S* alleles in the indigenous Gnaphalieae has not been investigated, but frequency-dependent selection is expected to preserve allelic variation and rare alleles over time (Richman and Kohn, 2000) and in the Solanaceae alleles possessed by different species or genera can be more similar than different alleles present in a single species (Ioberger *et al.*, 1990). If radiation is rapid and recent, as is implicated for the New Zealand Gnaphalieae (see Breitwieser *et al.*, 1999), sharing of *S* alleles by different, recently evolved taxa might be possible.

Generic relationships among the indigenous Gnaphalieae

Stuessy (1990 p. 201) advocated the value of cross-compatibility data to "indicate the *degree* of genetic cohesiveness of taxa which come from a single evolutionary line" and thus to test hypotheses of relationships based on structural data, such as morphology. However, taxonomic and phylogenetic interpretation of cross-compatibility data requires some caution. The ability to interbreed does not necessarily correspond with groups based on morphological or ecological criteria, and various internal post-pollination barriers can prevent closely related plants from hybridising (see Grant, 1981 pp. 111–117). Unsuccessful crosses involving alpine species might be due to environmental variables unfavourable for pollination or fertilisation at low altitudes. Cross-compatibility data can only be interpreted with confidence if all possible reciprocal crosses are performed and with adequate replication, which may be impracticable for large groups such as the New Zealand Gnaphalieae. Nevertheless, compatible artificial crosses provide

evidence that many indigenous Gnaphalieae from different genera are closely related (Figure 6.1 A p. 309) and that numerous species lacking the opportunity to hybridise in the field are cross-compatible. The results support ITS sequences (Glenny & Wagstaff, 1997; Breitwieser *et al.*, 1999), morphology (Ward, 1993), flavonoids (Breitwieser and Ward, 1993), and pollen characteristics (Breitwieser and Sampson, 1997a; Breitwieser and Sampson, 1997b) in suggesting indigenous *Anaphalioides*, *Ewartia*, *Helichrysum*, *Leucogenes* and *Raoulia* are closely related. This assemblage is hereafter referred to as the 'New Zealand endemic group'. Artificial crosses also strongly implicated a genetic affinity exists between *Euchiton* species and this assemblage. The results agree with ITS sequence data (Breitwieser *et al.*, 1999) in suggesting genera among the New Zealand Gnaphalieae have a higher genetic similarity than suggested by morphology, but additional replication and investigation of a wider range of species is required to help to resolve species relationships and generic delimitation within the New Zealand Gnaphalieae in future. The generic groupings of Merxmüller *et al.* (1977) and the subtribal classification of Anderberg (1991) are not supported, as cross-compatible genera are divided between groups or subtribes. Artificial hybrids are recorded between subtribes in the Gramineae and between subfamilies in the Orchidaceae (Stace, 1975 p. 13), but no such hybrids are recorded among other Compositae. The size of the Gnaphalieae (over 180 genera) means cross-compatibility data is unlikely to make a direct contribution to resolution of a satisfactory subtribal classification across the entire tribe, at least not in the short term, but such data are likely to prove valuable to test hypotheses of generic relationships within the tribe.

Many of the intergeneric hybrids studied in this thesis appeared to be partially fertile, providing additional evidence for a close genetic affinity between the parental genera. Some authors (e.g., Powell, 1985) believe intergeneric hybrids should be sterile or of low fertility. However, partially fertility appears to be more common than absolute sterility among intergeneric hybrids in the Compositae (e.g., Mitsuoka and Ehrendorfer, 1972; Kyhos *et al.*, 1990; Christov and Panayotov, 1991; Hartman and Lane, 1991; Vassilevska-Ivanova *et al.*, 1996). In the present study only two hybrids were suggested to be pollen sterile and certain hybrids (e.g., the *H. intermedium* var. *tumidum* × *A. bellidioides* hybrid 'Graeme Paterson' and a putative *H. intermedium* × *H. lanceolatum* hybrid) possessed more than 80 % normal pollen grains, but further experimentation is needed to determine whether the pollen grains are capable of achieving *in vivo* fertilisation. Experimental crosses demonstrated partial pollen and ovule fertility in a number of intergeneric hybrids (Figure 6.1 B p. 310), e.g., *A. bellidioides* × *H. lanceolatum* and *A. bellidioides* × *E. sinclairii* hybrids. The putative *H. intermedium* × *H.*

lanceolatum hybrid might be a later-generation hybrid. Hybrids have been collected from the same locality (Ghost Creek, Torlesse Range, Canterbury) by George Simpson (CHR 108600, no collection date specified) and in 1933 by H. H. Allan (CHR 108595), so there has been ample time for further interbreeding and backcrossing in the intervening years. As an indication of the rapidity with which full fertility can arise in intergeneric hybrids in the Compositae, Carr (1995) selected a fully fertile backcross hybrid derived from a putative F_1 hybrid between *Argyroxiphium sandwicense* subsp. *macrocephalum* and *Dubautia menziesii* of low fertility. The partial fertility of the intergeneric Gnaphalieae hybrids presents an exciting opportunity to investigate chromosome homology and genome evolution in the group through the synthesis of artificial hybrids in future. Such work may be valuable for elucidating the evolutionary history of the New Zealand Gnaphalieae, which in turn should aid resolution of taxonomic relationships. Artificial hybridisation would also be important for testing hypotheses of hybrid speciation and introgression, and for investigating whether past hybridisation events have contributed to the evolution of the indigenous Gnaphalieae.

Cross-compatibility of indigenous and exotic Gnaphalieae

Cross-compatibility results were consistent with ITS sequence data in suggesting that members of the New Zealand endemic group are more closely related to each other than to *Anaphalis*, *Antennaria*, *Ewartia meredithae*, *Leontopodium*, *Ozothamnus* and *Pseudognaphalium* (Breitwieser *et al.*, 1999). Anderberg (1991) suggested a close relationship for *Anaphalis* and *Anaphalioides*, and between *Leucogenes* and *Leontopodium*, but no crosses attempted between plants of these genera were compatible. However, more extensive replication is needed to confirm the incompatibility of these genera. Filled cypselas were obtained in crosses between plants of *Euchiton* with *Ewartia planchonii* and *Gamochaeta spicata*. In many incompatible crosses between plants of indigenous and exotic Gnaphalieae, pollen germination on the stigma occurred but no filled cypselas developed. Cross-compatibility with exotic taxa has been reported in a number of indigenous plants (e.g., Brockie, 1966; Nordenskiöld, 1971; Raven and Raven, 1976; Connor, 1983) and is consistent with the hypothesis of rapid, recent radiation within the New Zealand flora (see Winkworth *et al.*, 1999 and references therein).

Conclusions

Among indigenous Gnaphalieae, a high level of self-compatibility occurred in plants of five *Euchiton* species and two *Anaphalioides* species. Further experimentation is needed to determine the extent of self-compatibility at the individual, population and species levels. Differing levels of autonomous selfing in *Euchiton* and *Anaphalioides* reflect their contrasting capitulum

morphology and degree of adaptation for inbreeding. Artificial crosses provided evidence for a close relationship among indigenous *Anaphalioides*, *Ewartia*, *Helichrysum*, *Leucogenes* and *Raoulia*. Members of this group are cross-compatible and interfertile, despite considerable morphological and ecological radiation. A number of post-pollination (both pre- and post-zygotic) barriers and non-reciprocity in cross-compatibility appear to restrict hybridisation. Partial fertility was common among the intergeneric hybrids studied and therefore considerable potential for gene exchange between genera exists. However, additional experimentation is needed to interpret the taxonomic and evolutionary significance of the results.

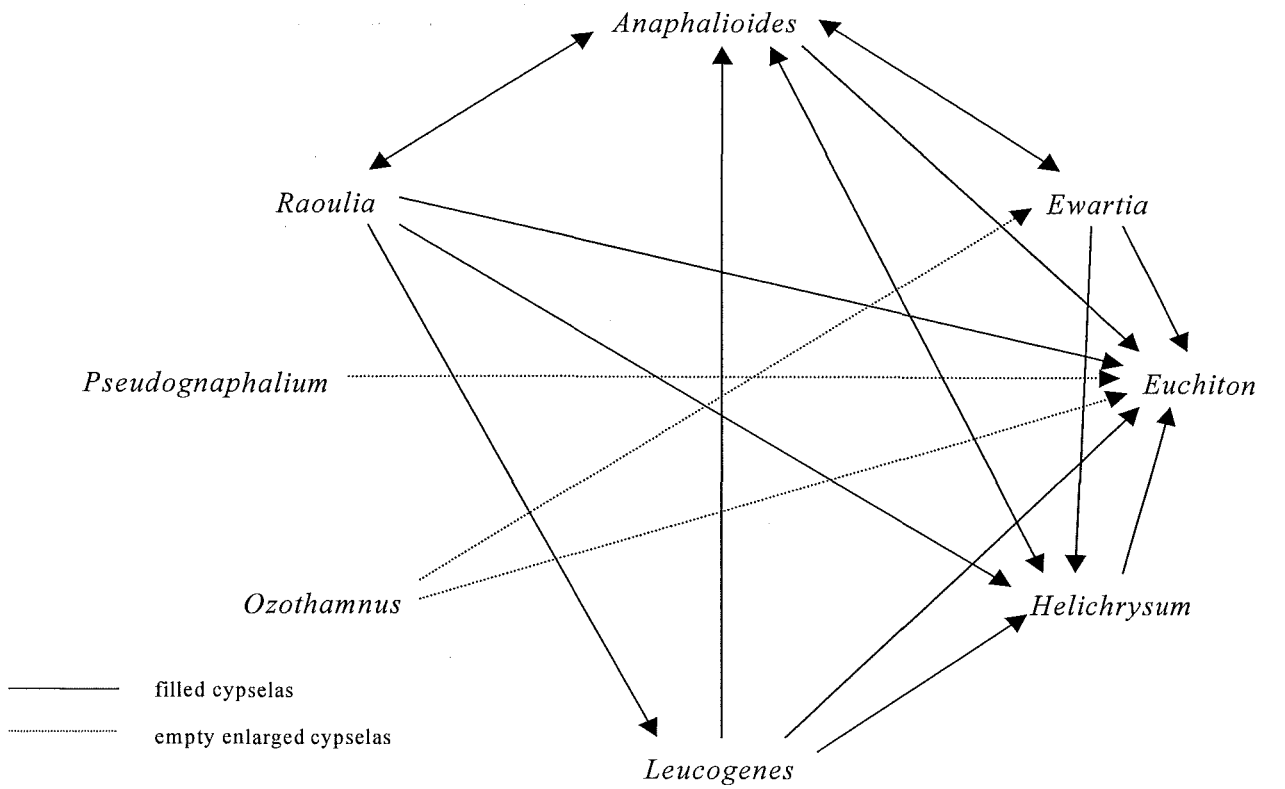


Figure 6.1. Cross-compatibility among indigenous Gnaphalieae, based on the results experimental crosses performed in this thesis. Arrows point to the maternal parent.

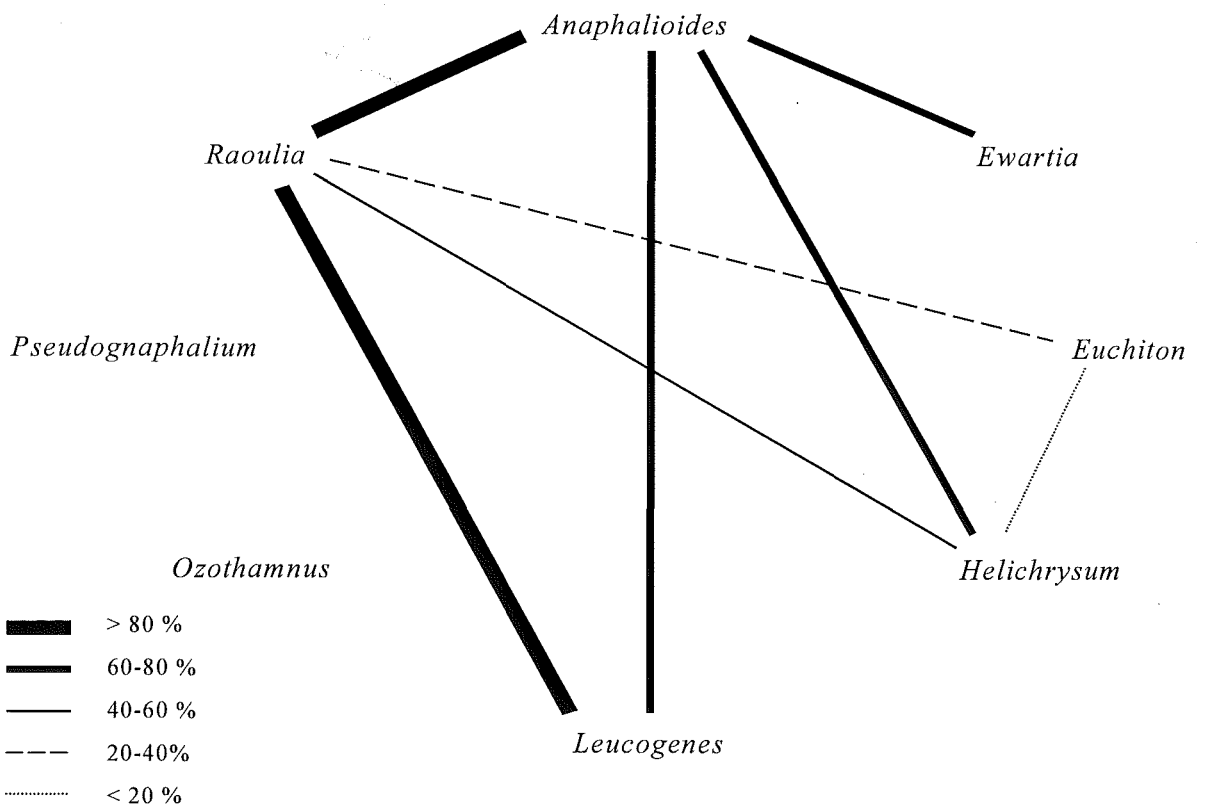


Fig. 6.2. Maximum pollen stainability of artificial and natural putative hybrids among indigenous Gnaphalieae, based on results from this thesis.

SECTION FOUR

General Discussion

Chapter 7. General Discussion

7.1 Identification of hybrids from morphological data

Evidence for hybridity and discrimination of groups of hybrids was obtained from morphology in the case studies. Previous studies of putative intergeneric hybrids among New Zealand Gnaphalieae have found morphology to be similarly informative (Jordan, 1995; Falvey, 1996). Identification of hybrids among the New Zealand Gnaphalieae from morphology is made difficult by the character distribution in the group, as species often possess various combinations of diagnostic generic character states (Breitwieser and Ward, 1993). The case studies demonstrated the importance of accessing a wide range of vegetative and floral, and continuous and discrete, characters to avoid misidentification of putative hybrids or the parental species. Continuous and discrete characters suggested differing relationships among the putative hybrids and sympatric species, as illustrated by character indices and metric multidimensional scaling (MDS) of data recorded from field-grown specimens in case study 1. The different analytic methods used also often suggested differing relationships, especially canonical discriminant analyses (CDA), the results of which varied depending on the composition of the data set.

In both case studies, most field-growing putative hybrids possessed a similar frequency of intermediate and parental character states, extreme character states were rare and novel characters absent. Novel characters were recorded in only two specimens (CHR 385817, a putative hybrid between *A. bellidioides* and *E. sinclairii* from the upper Hodder valley, and *S1*, a putative backcross to *A. bellidioides* or later-generation hybrid between *A. bellidioides* and *E. sinclairii*). A survey of 32 artificial F_1 hybrids by Rieseberg and Ellestrand (1993) indicated the pattern of morphological character expression is variable and not a reliable universal criterion for hybridity. In some F_1 hybrids surveyed the frequency of parental and intermediate characters were similar, but in other examples parental or intermediate characters predominated. In the light of this survey, the similar frequency of intermediate and parental characters and the limited variation among the field-growing putative hybrids in the present study, and the clear discrimination of the seed-raised and field-growing putative hybrids between *Anaphalioides bellidiodies* and *Ewartia sinclairii*, are thus not inconsistent with the suggestion that most of the field-growing putative hybrids were either F_1 hybrids or later-generation hybrids with a similar phenotype to F_1 hybrids. Synthesis of artificial hybrids and comparison with the field-collected putative hybrids would provide information on the genetic regulation of phenotypic character expression and the possible contribution of effects such as dominance, heterosis and matrocliny

and would allow more confident characterisation of the putative hybrids (as F_1, backcross or later-generation hybrids) from morphology, as noted by previous authors (e.g., Baker, 1947). Molecular genetic techniques, especially microsatellite markers and isozyme analysis, are now common and powerful tools for detecting hybridity and establishing genealogy in hybrids (see Rieseberg and Ellstrand, 1993). Such methods were beyond the scope of this thesis, but application of these techniques to New Zealand Gnaphalieae in future would permit characterisation of wild putative intergeneric hybrids with greater precision.

In both case studies, marked differences occurred in character expression between continuous and discrete characters in the putative hybrids. Predominantly intermediate states occurred for continuous characters and parental states for discrete characters. The greater frequency of parental states in discrete characters reflected constraints in satisfactorily coding more than two states for some characters, but at least some of the characters might be under simple (rather than polygenic) control. As noted by McDade (1997), the value of including parental characters in data sets to strengthen the linkage between hybrids and their parents was also demonstrated. Continuous characters were more informative regarding the degree of intermediacy of the putative hybrids, but appeared to be less reliable indcators of relationship when recorded from field-grown specimens. Mixed data sets were the most supportive of the hybridity hypotheses. The contrasting relationships suggested by continuous and discrete characters were well illustrated by character indices and HYWIN analyses. A similar pattern of character expression was exhibited in previous investigations of hybridisation among New Zealand Gnaphalieae (Jordan, 1995; Falvey, 1996). Continuous characters recorded from field-collected specimens tended to be more variable than those recorded from cultivated plants and were less supportive of the hybridity hypothesis for some putative hybrids (e.g., the putative hybrids between *A. bellidioides* and *E. sinclairii*, *W5* and *W12*). This might reflect the influence of environmental conditions on the morphology of field-growing plants. The greater frequency of extreme character values in cultivated putative hybrids, than in field-grown putative hybrids, between *A. bellidioides* and *E. sinclairii* suggested heterosis was expressed in certain continuous characters.

The exclusion from the data sets of characters that poorly discriminated species is likely to have enhanced resolution of the putative hybrids' relationships. Inclusion of characters varying independently of hybridity adds 'noise' to a hybrid index and blurs underlying patterns in the data set (Hatheway, 1962). Characters invariant within all species must be excluded for CDA, even though they might have a unique state for each species and thus be perfect discriminators (Sneath and Sokal, 1973), as the method uses within-group variances to optimise group discrimination.

CDA is very sensitive to characters containing outliers, which can greatly affect a variance estimate and classification accuracy (Huberty, 1994, pp. 94-96; McLachlan, 1992, pp. 181-185), but the impact of such characters on CDA in this thesis depended on their discriminatory power and the other characters included in the analysis. The presence of outliers did not affect the classification accuracy of the discriminant functions in case study 2, as specimens were always correctly classified by plug-in classification and crossvalidation. In case study 1, exclusion of characters with outliers reduced the misclassification rate, but at least one specimen was still misclassified, showing characters with outliers were not the sole factor contributing to misclassification.

In this thesis leaf anatomy was helpful for identification of the likely parental species, as in previous studies of putative intergeneric hybrids among New Zealand Gnaphalieae (Jordan, 1995; Falvey, 1996). The degree of mesophyll differentiation was useful in both case studies, and the presence and distribution of sclerenchyma fibres provided evidence for an affinity between *R. eximia* and some putative hybrids with *L. grandiceps*. Some variation in leaf anatomy among the putative hybrids was evident, but difficulties of coding intermediate discrete character states restricted the use of this variation in characterising the putative hybrids.

Detection of hybridity and identification of the most likely parental species was hindered by the presence of sympatric species morphologically similar to one of the putative parents (*Ozothamnus leptophyllus* in case study 1 and *Raoulia mammillaris* in case study 2). Reduction in the number of individuals in the data set, by excluding the least likely parental species, aided discrimination of individuals by CDA and MDS and usually increased the proportion of variation explained by the first and second axes. This is because the most dissimilar individuals were separated on the first dimension, so usually a second or third dimension was required to separate individuals of high similarity. McDade (1997) reported increased stress values with increasing number of hybrids in the data set in MDS.

Support for hybridity from analytic methods

Overall, the analytic methods used were strongly supportive of the hybridity hypothesis in case study 1 and consistently discriminated the seed-raised putative hybrids between *Anaphalioides bellidioides* and *Ewartia sinclairii*. The same methods were less conclusive with respect to identifying putative hybrids between *Leucogenes grandiceps* and *Raoulia eximia*. This most likely reflects differences in the nature of the data, differing relationships among the putative

hybrids, and differing degrees of similarity between parental and non-parental species in the two case studies.

The variable placement of the putative hybrids by different analytic methods in this thesis is not unexpected, since the methods operate on different types of data (distance data, continuous characters, or continuous and discrete data) and use very different means to represent relationships. Metric multidimensional scaling (MDS) and canonical discriminant analysis (CDA) provided the strongest support for the hybridity hypotheses, but all methods were informative regarding relationships among the putative hybrids. Similar groupings among the putative hybrids were suggested by some methods (e.g., character indices and cluster analysis in case study 1), but more often the relationships suggested by the different methods varied. Certain analyses (e.g., some HYWIN analyses) strongly contradicted the hybridity hypotheses, but the use of a multiple-method approach provided a form of cross-validation of the outcomes. Other comparative studies have shown different analytic methods can vary in their ability to detect hybridity and identify parental species (e.g., Neff and Smith, 1979; Pimentel, 1981; Adams, 1982; Brochmann, 1987; McDade, 1997).

The simplest numerical techniques (character counts and character indices) revealed the morphological intermediacy between the hypothesised parental species for all of the putative hybrids. As pointed out by Wilson (1992), intermediacy in hybrid indices can result from different character patterns, but the extremely low frequency of extreme and novel character states in the putative hybrids was suggestive of a hybrid origin for the specimens in the present study. The marked differences in the distribution of character states between continuous and discrete characters suggested differing hypotheses concerning the structure of the hybrids group. Both methods discriminated the seed-raised and field-growing putative hybrids between *A. bellidioides* and *E. sinclairii.*

In both case studies, CDA and MDS usually placed the putative hybrids intermediate or proximate to the putative parental species on the first dimension. In some instances putative hybrids were non-intermediate on the second and third axes, which were more informative with respect to characterisation of the putative hybrids. The non-intermediate placement of putative hybrids was not always consistent with their possession of extreme or novel characters. Adams (1982) found that putative hybrids with such characters were placed unpredictably by CDA. Both methods sometimes placed putative hybrids closer to non-parental than parental species,

such as when the intervening discriminant or phenetic space between parental species is occupied by other species. This has been noted in previous studies (e.g., McDade, 1997).

The placement of the putative hybrids by CDA tended to vary with different data sets, reflecting differences in the characters receiving high weighting, and was not always consistent with relationships suggested by other methods (e.g., the placement of the putative hybrid between *A. bellidioides* and *E. sinclairii*, *W 5*, by CDA and MDS). The weighting of characters in CDA also ensures a small number of characters will strongly influence the outcome, so character selection is critical in CDA.

The putative hybrids were occasionally predicted to belong to species other than the hypothesised parental species by CDA. For example, the putative hybrid between *A. bellidioides* and *E. sinclairii W12* was classified as *H. parvifolium*, and the putative hybrid between *L. grandiceps* and *R. eximia W5* was predicted to belong to *R. subsericea*. In such cases a non-parental species occupied discriminant space between the putative parental species (e.g., see Figures 4.5 p. 123 and 5.16 p. 242). However, scatter plots of the first three canonical variates did not account for some group predictions in certain analyses (e.g., the analysis represented by Figure 5.16). Inclusion of non-normal characters had little impact on group assignment for putative hybrids between *A. bellidioides* and *E. sinclairii*, but predictions were affected for putative hybrids between *L. grandiceps* and *R. eximia*. In all discriminant analyses performed in this thesis, covariance matrices were suggested to be highly homogeneous.

The goodness of fit of the MDS ordinations was high, as demonstrated by the low stress values. MDS has been advocated by some authors (e.g., Pimentel, 1981; Adams, 1982), but Brochmann (1987 p. 628) concluded the method "was unfitted as a final structure analysis. It was, however, appropriate for revealing dimensionality and parentage". Some comparisons of ordination methods (e.g., Pimentel, 1981; Minchin, 1987) have found MDS to be the most 'robust' technique; that is, it is best able "to recover an underlying Euclidean ordination space from data that do not fit a simple linear model of responses but may be highly skewed or noisy or show uneven responses in different parts of the space" (Crisp and Weston, 1993 p. 59).

The clustering of the putative hybrids and their linkage to one of the hypothesised parental species with hierarchical clustering is consistent with the results of other studies (Heiser *et al.*, 1965; Bemis *et al.*, 1970; McDade, 1997). This outcome is not surprising, since these methods aggregate or divide a set of individuals in a stepwise manner to produce groups of similar

individuals; at each step the most similar or dissimilar individual is linked or separated. The method is therefore severely constrained in how dissimilarities are visualised and is not ideally suited to representing hybrid intermediacy. The phenograms returned by hierarchical clustering had high overall correlation coefficients (greater than 0.85 for both Pearson's and Spearman's correlation coefficients), showing they were good representations of the dissimilarities overall. However, the placement of highly similar specimens varied following randomisation of the specimen order and correlation coefficients were lowest among terminal linkages. Consequently, ccluster analysis was more informative with respect to identifying possible *groups* among the putative hybrids. In all phenograms the putative hybrids formed a single cluster and none were suggested to be most similar to a non-parental species, unlike some CDA, MDS and HYWIN analyses.

In this thesis HYWIN was of greatest value for hypothesis testing. Adjusting the weightings provided information on the degree of intermediacy of the putative hybrids between either parental species. The results were strongly influenced by the composition of the data set. HYWIN was inefficient when analysing two- and three-state discrete characters and was better suited to analysing continuous data. In both case studies, discrete characters were excellent indicators of parentage, whereas continuous characters were more informative with respect to intermediacy between the hypothesised parents. Consequently, in this thesis HYWIN performed best when analysing mixed data. Previous studies employing HYWIN have analysed mixed data (Gil-ad and Reznicek, 1997; Tortosa *et al.*, 2000) or continuous data (Estabrook *et al.*, 1996), but previously discrete characters have not been analysed separately. When the putative parents were relatively similar and more dissimilar species were included in the data set, as in case study 2, a low parental distance was required to avoid erroneous hypotheses being highly ranked. If other species were intermediate or close to one parent, as in both case studies, high ranking of erroneous hypotheses appeared to be unavoidable. Restricting the number of species in the data reduced the number of alternative parentage hypotheses that were highly ranked. A further limitation of HYWIN is that hybrids must be intermediate between the parents, so non-intermediate hybrids and those with an higher frequency of extreme and novel characters are unlikely to be ranked among the most probable hypotheses. Some discrepancies between the results of HYWIN and discriminant analyses have been noted in other studies (Gil-ad and Reznicek, 1997; Tortosa *et al.*, 2000). Gil-ad and Reznicek (1997) considered HYWIN analyses provided "fine-tuning" where confident identification of a particular specimen was not possible with discriminant analysis. However, HYWIN did not fill such a role in this thesis.

In splits graphs generated in both case studies, the putative hybrids (especially between *L. grandiceps* and *R. eximia*) formed an ill-resolved group lacking an internal edge distinguishing the group and were always placed near the centre of the splits graph closely associated with the putative parental species. Except for *R. mammillaris* in case study 2, the nonparental species were separated by relatively long internal edges. These results might enable identification of hybrids and the putative parents in a splits graph. However, in certain analyses in case study 1 (e.g., compare Figure 4.11 C & E p. 128), splits graphs of similar structure were generated following sequential exclusion of species from the data set, suggesting some caution in interpretation of splits graphs is required when investigating hybridity. Split decomposition discriminated the field-collected and seed-raised putative hybrids between *A. bellidioides* and *E. sinclairii*, but groups among the putative hybrids between *L. grandiceps* and *R. eximia* were less resolved. In the only other published studies of plant hybrids to have utilised split decomposition, hybrids tended to be placed close to at least on of the parents (Ahmad *et al.*, 1996; Ahmad *et al.*, 1997; Hollingsworth *et al.*, 1999), but investigating the predictability of placement of hybrids in splits graphs was beyond the scope of these studies.

Of the three methods for analysing dissimilarities utilised in this thesis (cluster analysis, MDS and split decomposition), MDS provided the strongest support for hybridity. All three methods were informative with respect to relationships among the putative hybrids but sometimes suggested conflicting relationships, in part reflecting distortion and the very different means of visualising dissimilarities for each method. MDS and split decomposition tended to emphasise high dissimilarities and so the results were influenced by the number and nature of the species included in the data set. However, in both methods subsetting of the data set (e.g., including only the putative hybrids or sequentially excluding species) improved resolution of relationships among individuals of high similarity. Overall, cluster analysis, MDS and split decomposition were complimentary, but opinions on the relative merit of clustering and ordination techniques are often polarised (e.g., Sneath and Sokal, 1973 p. 201; Stuessy, 1990; de Queiroz and Good, 1997).

7.2 Barriers to hybridisation of indigenous Gnaphalieae and hybrid fertility

Experimental crosses demonstrated the potential for F_1, backcross and later-generation hybridisation among New Zealand Gnaphalieae genera. However, in both case studies natural hybrids were rare, the morphology of most field-growing plants was consistent with their being F_1 hybrids or phenotypically similar to F_1 hybrids, and all putative hybrids examined appeared to be partially fertile. The seed-raised hybrids between *A. bellidioides* and *E. sinclairii* were

concluded to comprise two backcrosses to *A. bellidioides* and either a later-generation hybrid or backcross to *A. bellidioides* (see p. 186), and provided evidence that hybrids beyond the first generation are produced in the field. Collectively, the results suggest the frequency of natural hybridisation is much reduced from the potential level, at least between *A. bellidioides* and *E. sinclairii*, and that various reproductive barriers may be restricting hybridisation in the field. Grant (1981 pp. 111-117) classified barriers to plants' hybridisation into three main classes (spatial, environmental and reproductive), each of which may be important to varying degrees in the New Zealand Gnaphalieae, as well as other Compositae (e.g., Sundberg and Stuessy, 1990). Many of the cross-compatible indigenous Gnaphalieae are geographically, ecologically or phenologically isolated (e.g., Allan, 1961; Wilton, 1997), ensuring that hybridisation under natural conditions is unlikely. The flowering periods of some sympatric Gnaphalieae overlap (Wilton, 1997) and some of these species (e.g., *A. bellidioides* and *L. grandiceps*) were shown to be artificially cross-compatible in this thesis. Herbarium-specimen labels and other authors (e.g., Allan, 1961) indicate natural intergeneric hybrids are sporadic only, but a possible exception is hybrids between *A. bellidioides* and *H. lanceolatum*. Cockayne and Allan (1926) referred to a polymorphic hybrid swarm between these species near Hanmer Springs and Webb (1988 p. 251) described this combination as "widespread and common". Williams (1989) listed six putative intergeneric hybrids among Gnaphalieae recorded from the Hodder valley by Dr B. P. J. Molloy, of which all were described as uncommon. The low frequency of natural hybrids in the two case studies indicates the parental species are not converging, despite the considerable potential for intergeneric gene exchange, but these rare hybrids should not be considered evolutionarily irrelevant. Studies of other plant and animal hybrids have shown that extremely low fertility or viability in early-generation hybrids, such as F_1, F_2 and first-backcross generations, does not preclude the establishment of later generations or new evolutionary lineages (Stace, 1993; Arnold *et al.*, 1999), and even rare hybridisation events might be evolutionarily important (Ellstrand *et al.*, 1996).

A variety of generalist insect pollinators have been observed visiting indigenous Gnaphalieae (Primack, 1983; Wilton, 1997). Some differences between species were recorded, suggesting ethological isolation might prevent hybridisation in some instances. No floral visitors to *Euchiton audax* and *E. traversii* capitula were observed by Wilton (1997), so a paucity of insect visits and adaptations for inbreeding might be the major isolating mechanisms for *Euchiton* species in the field. Differences in flowering phenology appear to be important for riverbed *Raoulia* species (Wilton, 1997), especially early and late flowering species such as *R. tenuicaulis* and *R. glabra*, but seed set was low or absent in artificial crosses between plants of the early

flowering species *R. haastii*, *R. monroi* and *R. tenuicaulis*, suggesting internal post-pollination barriers might also be a factor in their reproductive isolation. The contribution of ecological specialisation among species in providing spatial isolation would depend on the area serviced by pollinators.

Most studies of internal post-pollination barriers to intergeneric hybridisation in other Compositae have focussed on chromosomal differences (e.g., Mitsuoka and Ehrendorfer, 1972; Carr and Kyhos, 1986; Carr *et al.*, 1996). In the present study observation of post-pollination events indicated the following factors contribute to cross-incompatibility in the New Zealand Gnaphalieae: failure of pollen germination on the stigma; failure of style retraction or cypsela enlargement despite pollen germination (suggesting absence of fertilisation or early zygotic abortion); and development of empty cypselas (suggesting post-zygotic abortion of the embryo and/or endosperm). As already discussed (see pp. 321–322), artificial crosses suggested reciprocal differences in cross-compatibility might be important in some instances. Further experimentation is required to establish self-incompatibility (SI) among the New Zealand Gnaphalieae and evaluate the involvement of the SI system in cross-incompatibility.

Even if filled cypselas develop in an intergeneric cross, numerous factors may hinder the survival of the hybrids in the field and limit their capacity to reproduce. Germinability was high among the seeds sown from experimental crosses, but low inherent seed viability might be a factor in some crosses. Low hybrid fitness might affect the ability of hybrids to attain maturity and restrict the phenotypes able to survive in the field, as suggested for *L. grandiceps* × *R. eximia* hybrids (see p. 278–279). Hybrid inviability, defined by Grant (1981 p. 115) as consitutional weaknesses that block gene exchange between species in the vegetative phase of the F_1 generation, is recorded in some Compositae hybrids (e.g., Hollingshead, 1930). Limited availability of suitable habitats and natural disturbance might hinder establishment of hybrid seedlings at the study sites, especially given the slow growth rate of *R. eximia* and *L. grandiceps* × *R. eximia* hybrids. Prepotency (see Schoen and Lloyd, 1992) and reduced fertility of the hybrids might also limit the capacity of mature hybrids to reproduce.

Intergeneric hybrid fertility

Reduction in fertility is not an absolute indicator of hybridity (Stace, 1986) and is not correlated with taxonomic distance. Interspecific hybrids can be absolutely sterile or as fertile as either parent (Stace, 1989). Artificial F_1 hybrids between *Helianthus annuus* and *H. petiolaris* have pollen fertility of 0–30 %, and F_2 and backcross progeny produce only 1–2 % viable seed (see

Arnold *et al.*, 1999). The fertility of intergeneric F_1 hybrids in the Compositae is variable. For example, pollen stainability (supplemented by meiotic pairing data) in F_1 hybrids between *Chrysanthemum coronarium* L. and *Ismelia carinata* Sch.Bip. was 19–84 % (Chaudhuri *et al.*, 1976). A fully fertile backcross hybrid between *Argyroxiphium sandwicense* subsp. *macrocephalum* and *Dubautia menziesii* was raised from putative F_1 hybrids of low fertility (Carr, 1995) and F_4 hybrids between *Helianthus annuus* and *Verbesina helianthoides* have been raised (Vassilevska-Ivanova *et al.*, 1996). Increased sterility or 'hybrid breakdown' can occur in the F_2 generation, e.g. through disruption of coadapted gene complexes (e.g., Clausen, 1951 pp. 109-111; Stebbins, 1958), but reports of absolute sterility in intergeneric Compositae hybrids (e.g., Kyhos *et al.*, 1990; Carr *et al.*, 1996) are uncommon.

In the present study, artificial crosses involving putative hybrids, pollen stainability and meiotic pairing provided evidence for reduced fertility in most of the putative intergeneric hybrids examined. Pollen stainability ranged from zero (in an experimental hybrid between *Euchiton* cf. *involucratus* and *H. intermedium*, and a putative hybrid between *A. bellidioides* and *L. grandiceps* from Mt Hutt) to over 80 % (e.g., in a putative hybrid between *L. grandiceps* and *R. eximia*). Most putative hybrids exhibited a moderate level of pollen stainability, in common with the putative hybrids studied by Jordan (1995) and Falvey (1996). Further experimentation is required to determine the capacity of the putative hybrids' pollen grains to achieve *in vivo* germination and fertilisation, as both Alexander's differential stain and the fluorochromatic reaction have limitations and the results might not agree with germinability tests (Dafni and Firmage, 2000). Reduced pollen stainability in one plant of *R. eximia* might have been caused by resource limitations or detrimental environmental conditions during pollen development, and suggests greater sampling is required to determine the level of parental variation, at least in *R. eximia*.

Meiotic irregularities were at least partially responsible for the presence of abnormal pollen grains in putative *A. bellidioides* × *E. sinclairii* and *L. grandiceps* × *R. eximia*. The formation of micronuclei during meiosis, particularly in the putative hybrid between *A. bellidioides* and *E. sinclairii*, was consistent with the observation of tiny pollen grains with little or no stainable cytoplasm. Other irregularities observed comprised unpaired chromosomes (univalents) at metaphase I, lagging chromosomes at anaphase I and II, and occasionally bridges at anaphase I. It was not determined whether univalents resulted from asynapsis (absence of pairing between homeologous chromosomes during prophase I) or desynapsis (separation after pairing but prior to metaphase I), but the irregular distribution of univalents at metaphase I suggested asynapsis

was most likely. The univalents tended to be more widely separated in the *A. bellidioides* × *E. sinclairii* hybrid than in the *L. grandiceps* × *R. eximia* hybrid, suggesting the chromosomes in question had low attraction. An irregular number and distribution of the univalents during anaphase I and II resulted in a variable number of micronuclei in the microsporocytes. These irregularities have been observed in other intergeneric hybrids in the Compositae (e.g., Chaudhuri *et al.*, 1976; Kyhos *et al.*, 1990). Other meiotic irregularities reported in intergeneric Compositae hybrids include multivalents up to decavalents (Chaudhuri *et al.*, 1976), unpaired chromosomal segments at pachytene (Jackson and Dimas, 1981) and unstable karyotypes resulting in variable chromosome number in the same plant (Ono, 1951; Ono, 1955; Ono and Nagai, 1958).

The relatively high frequency of meiotic pairing observed in the putative hybrids studied in this thesis, especially the putative hybrid between *L. grandiceps* and *R. eximia*, was consistent with pollen stainability, but was unexpected given the divergent morphology of the parental species. Abnormalities were more frequent in the putative hybrid between *A. bellidioides* and *E. sinclairii* than the putative hybrid between *L. grandiceps* and *R. eximia* hybrid, suggesting higher chromosome homeology may exist between the latter two species than between *A. bellidioides* and *E. sinclairii*. Data on chromosome pairing are powerful indicators of genetic relationship. Hybrids between species differentiated primarily by genic factors tend to have regular meiotic pairing and high fertility, whereas hybrids between species with numerical or structural differences in chromosomes tend to have irregular pairing and usually reduced fertility (John and Lewis, 1965). Disruption of bivalent formation and of chromosome distribution at anaphase in hybrids might reflect chromosomal rearrangements in the parental species (see Grant, 1981 pp. 103-105). However, pairing is not an unequivocal measure of chromosome homology. For example, pairing between homeologous chromosomes may be genetically suppressed in hybrids, subsidiary associations of non-homologous chromosomes may occur, and the level of meiotic pairing and fertility in hybrids is not necessarily correlated (John and Lewis, 1965). The high frequency of pairing observed in the two putative hybrids is consistent with the percentage of pollen grains of normal appearance, but is surprising given the divergent morphology of the parental species.

7.3 Implications for generic concepts

As Rollins (1953 p. 134) highlighted, "In determining the nature and even the limits of a given genus, interest should centre upon the relatedness of the species." In this regard cross-compatibility and interfertility data are highly informative, but the implications of such data for

generic boundaries depends on the generic concept followed. Taxonomists have four alternatives when delimiting cross-compatible genera (Stuessy, 1990 p. 201): amalgamation of the genera, placement of the cross-compatible species in one of the genera, placement of cross-compatible species in their own genus, or no change in taxonomic position. Some authors consider cross-compatibility or interfertility between genera is critical when delimiting genera (Rollins, 1953; Löve, 1963; Powell, 1985). However, reproductive isolation does not necessarily accompany genotypic and phenotypic divergence, as exemplified by some insular Compositae (see Baldwin, 1998). Uniting cross-compatible genera might obscure rather than clarify the phylogeny of a group, as pointed out by Hartman and Lane (1991). In addition, as Stuessy (1990) highlighted, the performance of crosses between *all* species pairs, which is often impracticable, is required to fully understand the significance of cross-compatibility data. Therefore, cross-compatibility and hybrid-fertility data cannot be considered universal criteria for the delimitation of genera. Compatible crosses, however, provide strong evidence that two plants are genetically related, and information on the hybrids' fertility and chromosome homology provides information on the degree of relationship.

The present study supports previous investigations (Breitwieser and Ward, 1993; Ward, 1993; Breitwieser and Sampson, 1997a; Breitwieser and Sampson, 1997b; Wilton, 1997; Breitwieser *et al.*, 1999) in providing evidence for a close relationship among indigenous species of *Anaphalioides*, *Ewartia*, *Helichrysum*, *Leucogenes* and *Raoulia* (hereafter referred to as the 'New Zealand endemic group'). Cross-compatibility data also indicate *Euchiton* has a close genetic affinity with this group and support ITS sequence data (Breitwieser *et al.*, 1999) in suggesting the evolutionary lineages within the New Zealand endemic group are not as distinct as morphology suggests. For example, *Anaphalioides bellidioides*, *Ewartia sinclairii*, *Leucogenes grandiceps* and *Raoulia eximia* are very divergent in morphology but evidence for the existence of partially fertile hybrids involving these species has been presented in this thesis. *Anaphalioides bellidioides* and *Ewartia sinclairii* were briefly placed together in *Helichrysum* (Cheeseman, 1906; Kirk, 1899), but a broad generic concept for *Helichrysum* was accepted at the time and the genus was defined primarily by the female:hermaphrodite floret ratio. Since Cheeseman (1925) transferred *E. sinclairii* to *Ewartia*, no author has advocated *A. bellidioides* and *E. sinclairii* should be congeneric. *Leucogenes grandiceps* and *R. eximia* have never been considered to be congeneric, yet the low frequency of meiotic irregularities in a putative hybrid between the species demonstrates a close genetic affinity exists between the species.

Delimiting genera in the New Zealand endemic group from morphology is difficult owing to the overlapping distribution of diagnostic generic character states (Breitwieser and Ward, 1993). The cross-compatibility and hybrid fertility data, when considered in isolation, suggest generic limits should be broadened from those presently accepted. However, definition of the generic boundaries would be no easier. Including all cross-compatible or interfertile species in a single genus would preclude delimitation of coherent, well-defined genera and would conceal the extent of radiation that has occurred within the group. The cross-compatibility of indigenous *Euchiton* species with *Ewartia planchonii* and *Gamochaeta spicata*, which in turn may be cross-compatible with other genera, may make it difficult to delimit reproductively isolated genera in the Gnaphalieae. In other Compositae tribes there appears to be a general acceptance of some degree of intergeneric hybridisation (e.g., Powell, 1985; Carr, 1990; Hartman and Lane, 1991; Nesom, 1994). Recently, Baldwin (1999) segregated or reinstated seven genera in the Heliantheae in the quest for monophyly, even though a number of these genera are cross-compatible with others. Intergeneric cross-compatibility might be a widespread feature of the Gnaphalieae, not just the New Zealand members of the tribe, but until experimental crosses involving other genera are performed, it is difficult to draw taxonomic and evolutionary conclusions from the present results.

The results of the present study and previous investigations of natural hybrids (Jordan, 1995; Falvey, 1996) do not support the generic groupings of Merxmüller *et al.* (1977) nor the subtribal classification of Anderberg (1991). Acceptance of either classification would result in the occurrence of natural hybrids and experimentally cross-compatible species between the groups and subtribes (Figure 7.1 A & B p. 327). In the Gramineae artificial intertribal crosses (e.g., between *Bromus* L. and *Festuca* L.) may be successful (see Stace, 1975) and in the Orchidaceae members of different subfamilies may be cross-compatible (Solbrig, 1970), but neither type of hybrid is recorded in the Compositae. The cross-compatibility results agree with other types of evidence, including morphology (Ward, 1993), leaf flavonoids (Breitwieser and Ward, 1993) and ITS sequences (Breitwieser *et al.*, 1999), in suggesting Anderberg's subtribal classification is unsatisfactory, at least with regard to the New Zealand Gnaphalieae.

7.4 Comparison of New Zealand Gnaphalieae with other insular Compositae

The New Zealand Gnaphalieae exhibit considerable morphological diversity and include stoloniferous rosulate herbs (*Euchiton* species), mat plants (e.g., *Raoulia* subg. *Raoulia*), whipcord shrubs (e.g., *Helichrysum intermedium*), cushion shrubs (e.g., *Raoulia eximia*) and a divaricating shrub (*H. lanceolatum*). Different species occupy a wide variety of habitats,

including fellfield, forest margins, grassland, riverbeds, and scree. ITS sequences suggest the New Zealand Gnaphalieae are of recent origin and have evolved rapidly (Breitwieser *et al.*, 1999), but the present study shows that morphological and ecological radiation has not been accompanied by the development of barriers to hybridisation. In these respects the group is similar to certain other insular Compositae, such as Canary Island *Argyranthemum* (Brochmann *et al.*, 2000) and Hawaiian Compositae (Baldwin, 1998). In the Hawaiian silversword alliance, at least six changes in growth form are hypothesised and ecological changes are associated with radiation on different islands (Baldwin, 1998). Ornduff (1962) concluded from the results of artificial crosses between subspecies of the New Zealand species *Senecio glaucophyllus* that environmental adaptation was the primary evolutionary emphasis and that sterility barriers, at least between allopatric taxa, developed only "incidentally".

Hybridisation is considered to have contributed to the evolution of Canary Island *Argyranthemum* (Brochmann *et al.*, 2000), but natural hybridisation is relatively rare and considered of low evolutionary importance in the radiation of Hawaiian *Bidens* (Ganders and Nagata, 1984) and endemic Compositae (and other angiosperms) of the Juan Fernandez Islands (Stuessy *et al.*, 1998). Natural hybrids, some of them intergeneric and some forming hybrid swarms, are more frequent among the Hawaiian silversword alliance (Carr and Kyhos, 1986; Carr, 1990) and allopolyploidy between members of the North American tarweeds followed by long-distance dispersal is implicated in the origin of the Hawaiian silversword alliance (Barrier *et al.*, 1999). Hybridisation might be predicted to be of variable importance in the evolution of insular plants. For example, pollination limitations on islands might favour selection for adaptations promoting selfing or the evolution of sexual dimorphisms (Barrett, 1996). Increased selfing might restrict hybridisation, whereas sexual dimorphism promotes outcrossing and, potentially, hybridisation. Results from the present study and those of Wilton (1997) suggest at least some *Euchiton* species are adapted for delayed selfing and selection for functionally sexual dimorphism in *Raoulia mammillaris* might be occurring. The type of pollen vector (e.g., animal or wind, and generalist or specialist pollinator) and proximity between species will also influence the potential for natural hybridisation.

Baldwin (1998) suggested in Hawaii hybridisation might be of "episodic" importance, permitting new, stable recombinants to arise following environmental disturbance. Rattenbury (1962) argued that reproductive isolation has not developed in the New Zealand forest flora because selection has favoured groups that are interfertile and polymorphic. Island populations usually exhibit greater differentiation but contain less diversity than comparable continental populations

(Barrett, 1996). Therefore hybridism would be beneficial for the persistence of adaptive forms and maintenance of genetic variation, which would help small populations to survive environmental fluctuations and disturbance. However, Rattenbury's (1962) assumption of a high incidence of hybridism and interfertility in the New Zealand flora has still not been adequately demonstrated, and he acknowledged (p. 361) that one alternative hypothesis is that the polymorphy and interfertility represents recent adaptive radiation into newly available edaphic niches. Current evidence suggests evolutionary patterns among the indigenous Gnaphalieae are complex and past hybridisation events might not be easily detected. Nevertheless, the present study demonstrates intergeneric hybridisation has a potential role in the future evolution of the group.

7.5 Conclusions

This thesis provides morphological evidence for two instances of natural intergeneric hybridisation in the New Zealand Gnaphalieae: *Anaphalioides bellidioides* × *Ewartia sinclairii*, and *Leucogenes grandiceps* × *Raoulia eximia*. Many indigenous Gnaphalieae could be artificially crossed and partial fertility appears to be common in intergeneric hybrids. A variety of spatial, environmental and reproductive isolating mechanisms is suggested to restrict the opportunity for intergeneric hybridisation in the field. Thus the considerable morphological and ecological radiation in the group has not been accompanied by the development of barriers to hybridisation. This study also highlights the taxonomic value of studies of hybrids and of cross-compatibility, by providing insights into genetic affinity, evolutionary relationships and information on character expression.

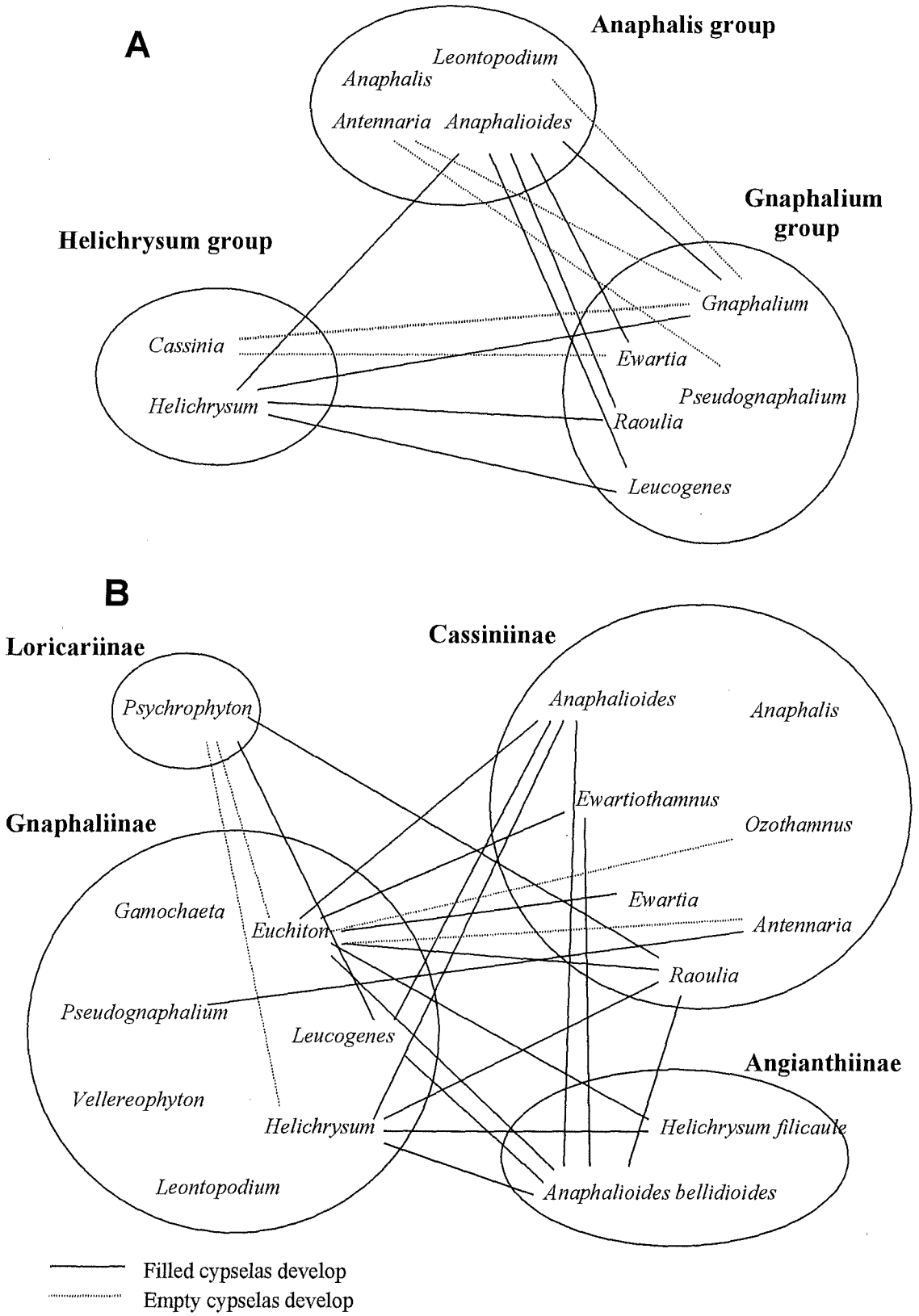


Figure 7.1. Comparison of artificial cross-compatibility between Gnaphalieae genera included in crosses in this thesis. Incompatible crosses are not indicated. Genera are arranged by: **A**, the generic groupings of Merxmüller *et al.* (1977); **B**, the subtribes of Anderberg (1991).

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Appendix 1. Split decomposition analysis

Split decomposition is a recently developed technique for analysing distance or sequence data (Bandelt and Dress, 1992b). A brief synopsis of the method and the construction of splits graphs is presented below (see also Page and Holmes, 1998).

Split decomposition transforms dissimilarities or sequence data into weighted 'splits'. For a quartet d of dissimilarities between pairs of taxa, there are three sums of the distances between each pair of taxa; for example, for four taxa a, b, c and d, the three distance sums are $d_{ab} + d_{cd}$, $d_{ac} + d_{bd}$, and $d_{ad} + d_{bc}$. The sum of the between-group distances can be expected to be larger than the sum of the within-group distances, but in practice split decomposition only assumes that for each group the sum of the within-group distances is at least not the largest of all sums collectively. The separation of ab and cd into two groups is termed a 'split'. The maximum number of splits for a $n \times n$ distance matrix is $\frac{n(n-1)}{2}$. A split system is 'compatible' if exactly one tree is supported for each quartet of OTUs. A 'weakly compatible' split system supports at most two trees for each quartet and so is less restrictive.

Every split receives a non-negative weighting value termed the 'isolation index'. Partitions that do not qualify as splits have an isolation index value of 0. Every split gives rise to a 'split metric' that assigns a distance value of 1 to two taxa from different groups and 0 otherwise. The sum d^I of all split metrics weighted by their isolation indices approximates d (Bandelt and Dress, 1992a). Comparison of the two matrices d and d^I gives a measure of the effectiveness of the split decomposition. The 'splittage percentage' indicates the average proportion of the given distances between taxa recovered from the weighted sum of split metrics. A residue, d^0, which is not decomposable into further splits with positive isolation indices, is usually reasonably small in real data sets (Bandelt and Dress, 1992b). The residue can be considered unresolvable 'noise' in the data and is 0 if $d = d^I$.

Construction of a 'splits graph' enables visualisation of the split metrics and their corresponding support in d^I (Bandelt and Dress, 1992b; Dress *et al.*, 1996). Each split is represented as a band of parallel and equal edges. Deleting all edges in a split divides the graph into two components. The length of the edges of a given split corresponds to the isolation index and indicates its weight or support. A splits graph enables visualisation of

conflicting patterns in the data by allowing a second dimension for internal edges rather than a single line. In general, a splits graph is more tree-like and less network-like when the number of analogies (sensu Hennig, 1966) are small and when patterns are more resolved and less contradictory (Lockhart *et al.*, 1995; Wagele, 1996).

The method assumes that triangle inequalities are satisfied (i.e., the distance d between any three points P, Q and R is such that $d(P, Q) \leq d(P, R) + d(Q, R)$). When this assumption is not met, an offset (e.g., 0.01) can be added to the dissimilarities.

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Appendix 2. Collection data and voucher details

Anaphalioides alpina (Cockayne) Glenny. **A**: Taranaki, Mt Egmont, D.Glenny 4094; **B**: Rangiwahia, D.Glenny 4203.

Anaphalioides bellidioides (G.Forst.) Glenny. **A**: Canterbury, Ahuriri River, A.D.Wilton 116; **B**: Wairarapa, Ruakokoputuna Valley, D.Glenny 4240; **C**: Campbell Island, D.Glenny 4493; **D**: Canterbury, Mt Hutt, J.M.Ward 95062; **E**: Canterbury, Mt Hutt, J.M.Ward 95063; **F**: Canterbury, Port Hills, R.J.McKenzie 102/1; **G**: Canterbury, Cass River, R.J.McKenzie 109/1-5; **H**: Canterbury, Ashley Gorge, R.J.McKenzie 130; **I**: Marlborough, Yeo Stream, R.J.McKenzie 139/1-3, 281/3-5; **J**: Canterbury, Ghost Creek, R.J.McKenzie 223/6.

Anaphalioides hookeri (Allan) Glenny. **A**: Otago, Shag Point, J.M.Ward 91335/2.

Anaphalioides trinervis (G.Forst.) Anderb. **A**: Nelson, Anatoki Valley, A.D.Wilton 243; **B**: Nelson, Aorere Valley, D.Glenny 4568; **C**: Westland, Costello Reserve, D.Glenny 4585; **D**: Westland, Lake Mapourika, I.Breitwieser 2036.

Anaphalis margaritacea (L.) Benth. & Hook.f. Cultivated plant from commercial nursery (provenance unknown). CANU 37506.

Antennaria dioica (L.) Gaertn. 'Rosea'. Cultivated plant from commercial nursery (provenance unknown).

Euchiton audax (D.G.Drury) Holub. **A**: Volcanic Plateau, Lake Moawhango, J.M.Ward 96012; **B**: Volcanic Plateau, Lake Moawhango, J.M.Ward 96015; **C**: Volcanic Plateau, Lake Moawhango, J.M.Ward 96031; **D**: Canterbury, Cass, R.J.McKenzie 226.

Euchiton cf. *involucratus* (G.Forst.) Holub. **A**: Kaimanawa Range, J.M.Ward 94209.

Euchiton delicatus (D.G.Drury) Holub. Volcanic Plateau, Lake Moawhango, J.M.Ward 96089.

Euchiton lateralis (C.J.Webb) Breitw. & J.M.Ward. Marlborough, near Lake Tennyson, G 41/96.

Euchiton limosus (D.G.Drury) Holub. **A**: Volcanic Plateau, Lake Moawhango, J.M.Ward 96018; **B**: Volcanic Plateau, Lake Moawhango, J.M.Ward 96019; **C**: J.M.Ward 96090.

Euchiton mackayi (Buchanan) Anderb. Marlborough, Balaclava Ridge, coll. G. N. Bawden.

Euchiton nitidulus (Hook.f.) Anderb. **A**: Marlborough, Balaclava Ridge; **B**: Canterbury, Cass Saddle, R.J.McKenzie 233/1-2.

Euchiton ruahinicus (D.G.Drury) Breitw. & J.M.Ward. **A**: Canterbury, Cragieburn Valley, R.J.McKenzie 248.

Euchiton sp. J.M.Ward 94036.

Euchiton traversii (Hook.f.) Holub. **A**: Canterbury, Cass, R.J.McKenzie 227/1; **B**: Canterbury, Kettlehole tarn, Cass, R.J.McKenzie 237; **C**: Marlborough, Barefell Pass, R.J.McKenzie 152.

Ewartia meredithae (F.Muell.) Beauverd. Tasmania, J.M.Ward 95158/3.

Ewartia planchonii (Hook.f.) Beauverd. **A**: J.M.Ward; **B**: J.M.Ward 94107/1; **C**: J.M.Ward 95157/1-3.

Ewartia sinclairii (Hook.f.) Cheeseman. **A**: Marlborough, Yeo Stream, R.J.McKenzie 140/1-5; **B**:

Marlborough, Yeo Stream, R.J.McKenzie 145; **C**: Marlborough, seed ex Hodder River, R.J.McKenzie 186/1.

Gamochaeta spicata (Lam.) Cabrera. Marlborough, Timms Valley, R.J.McKenzie 329.

Helichrysum depressum (Hook.f.) Benth. & Hook.f. **A**: Marlborough, Clarence Valley, R.J.McKenzie 163/1.

Helichrysum dimorphum Cockayne. Canterbury, Poulter River, coll. A.D.Wilton.

Helichrysum filicaule Hook.f. **A**: Banks Peninsula, J.M.Ward 84012; **B**: Wellington, Rimutaka Range, J.M.Ward 91213/2; **C**: Canterbury, Port Hills, R.J.McKenzie 101/1; **D**: Banks Peninsula, Stony Bay Peak, R.J.McKenzie 300/3.

Helichrysum intermedium G.Simpson. **A**: G 16608; **B**: G 16628; **C**: Canterbury, Mt Hutt, J.M.Ward 88216/3; **D**: Otago, Kurow, J.M.Ward 91262/1.

Helichrysum intermedium var. *tumidum* Cheeseman. **A**: Otago Peninsula, Sandymount, J.M.Ward 88339/1.

Helichrysum lanceolatum (Buchanan) Kirk. **A**: Banks Peninsula, Jubilee Road, coll. J.M.Ward; **B**: Canterbury, Port Hills, R.J.McKenzie 103/1-2; **C**: Banks Peninsula, Long Bay Rd, R.J.McKenzie 215/1-4.

Helichrysum parvifolium Yeo. Marlborough, Jollies Pass Reserve, J.M.Ward 96088.

Leontopodium palibinianum Beauverd. Cultivated plant from commercial nursery (provenance unknown).

Leontopodium sp. Cultivated plant from commercial nursery (provenance unknown), J.M.Ward 95075.

Leucogenes grandiceps (Hook.f.) Beauverd. **A**: Canterbury, Mt Hutt, R.J.McKenzie 183/1-6; **B**: Canterbury, Ohau, coll. A.D.Wilton.

Leucogenes leontopodium (Hook.f.) Beauverd. **A**: Wellington, Mt Holdsworth, coll. P.Salmond; **B**: Marlborough, Mt Patriarch, coll. P.Salmond.

Ozothamnus hookeri Sond. J.M.Ward 95139.

Ozothamnus leptophyllus (G.Forst.) J.M.Ward & Breitw. **A**: Fiordland, Scotts Basin, A.D.Wilton 310; **B**: provenance unknown; **C**: Wellington, Lyall Bay, coll. L.Baxter; **D**: Marlborough, Barefell Pass, R.J.McKenzie 162/7.

Ozothamnus rosmarinifolius. J.M.Ward 94122.

Pseudognaphalium luteoalbum (L.) Hilliard & B.L.Burtt. **A**: Volcanic Plateau, J.M.Ward 96016 (annual); **B**: Marlborough, Yeo Stream, R.J.McKenzie 146/2 (perennial); **C**: Canterbury, Cragieburn Valley, R.J. McKenzie 247/1; **D**: Canterbury, Ghost Creek, R.J.McKenzie 232/1-2 (perennial).

Pseudognaphalium luteoalbum var. *compactum* Kirk. **A**: Canterbury, Cass, Kettlehole Tarn, R.J.McKenzie 238/1-3.

Raoulia albosericea Colenso. **A**: Kaimanawa Range, J.M.Ward 94207/3; **B**: New Zealand, Volcanic Plateau, J.M.Ward 96021.

Raoulia apicinigra Kirk. **A**: A.D.Wilton 326; **B**: Marlborough, Balaclava Ridge; **C**: Canterbury, Mt Hutt, J.M.Ward 84005/2; **D**: Marlborough, Barefell Pass, R.J.McKenzie 161.

Raoulia australis Hook.f. **A**: J.M.Ward 90002; **B**: Canterbury, Kaitorete Spit, R.J.McKenzie 100/1-5; **C**, J. M. Ward 93017/2; **D**, R.J.McKenzie 134/1.

Raoulia beauverdii Cockayne. **A**: Otago, Otematata, J.M.Ward 90021; **B**: Otago, Lake Ohau, coll. A.D.Wilton; **C**: Otago, road to Lake Ohau, coll. J.M.Ward.

Raoulia bryoides Hook.f. **A**: Marlborough, Altimarlock, J.M.Ward 95084; **B**: R.J.McKenzie 372/1.

Raoulia eximia Hook.f. **A**: Canterbury, Mt Hutt, R.J.McKenzie 181/1-10.

Raoulia glabra Hook.f. **A**: Canterbury, Mt Hutt, R.J.McKenzie 246; **B**: Canterbury, Hawdon River, R.J.McKenzie 128.

Raoulia grandiflora Hook.f. **A**: Ohau, coll. A.D.Wilton 1/1/95; **B**: Canterbury, Mt Potts, coll. A.D.Wilton; **C**: Canterbury, Mt Hutt; RS/5.

Raoulia haastii Hook.f. **A**: Canterbury, Cass River, R.J.McKenzie 107/1-5.

Raoulia hookeri Allan. **A**: Marlborough, Wairau River, A.D.Wilton 166; **B**: R.J.McKenzie 225; **C**: Canterbury, Ahuriri River, A.D.Wilton 155; **D**: Marlborough, Wairau River, J.M.Ward 93016/2; **E**: Canterbury, Cass River, R.J.McKenzie 106.

Raoulia hookeri "Coast". Marlborough, Ward Beach, J.M.Ward 74056/6.

Raoulia mammillaris Hook.f. Canterbury, Mt Hutt, R.J.McKenzie 191/1-9.

Raoulia monroi Hook.f. **A**: Canterbury, Cass Flat, coll. A.D.Wilton; **B**: Canterbury, Lake Heron, J.M.Ward 88127; **C**: Canterbury, Port Hills, R.J.McKenzie 104/1-3.

Raoulia sp. "K". Volcanic Plateau, J.M.Ward 96005.

Raoulia sp. "M". Canterbury, Ryton Basin, J.M.Ward 90050.

Raoulia subsericea Hook.f. **A**: Canterbury, Mt Potts, coll. A.D.Wilton; **B**: Canterbury, Mt Terako, coll. A.D.Wilton; **C**: Awakino, coll. A.D.Wilton. **D**: Canterbury, Mt Hutt, R.J.McKenzie 172/1-6.

Raoulia tenuicaulis Hook.f. **A**: Canterbury, Mt Lyford, J.M.Ward 95072; **B**: Volcanic Plateau, Mt Ruapehu, J.M.Ward 96020/1; **C**: Volcanic Plateau, Mt Ruapehu, J.M.Ward 96020/3; **D**: Marlborough, Lake Chalice, coll. M.Todd; **E**: Canterbury, Cass River, R.J.McKenzie 119; **F**: Canterbury, Cass River, R.J.McKenzie 285; **G**: R.J.McKenzie 292/12; **H**: Marlborough, Rainbow Ski Area Road, R.J.McKenzie 450.

Raoulia youngii (Hook.f.) Beauverd. Canterbury, Mt Dobson, coll. S. Murray.

Vellereophyton dealbatum (Thunb.) Hilliard & B.L.Burtt. Tasmania, J.M.Ward 95141.

Natural putative hybrids:

Anaphalioides bellidioides × *Anaphalioides trinervis*. Westland, Fox Glacier - Franz Josef Road, coll. A.D.Wilton.

Anaphalioides bellidioides × *Helichrysum lanceolatum*. **A**: Canterbury, Port Hills, J.M.Ward 93093/5; **B**: Banks Peninsula, Long Bay Road, coll. R.J.McKenzie.

Anaphalioides bellidioides × *Ewartia sinclairii*. See pp. 85–86.

Anaphalioides bellidioides × *Leucogenes grandiceps*. Canterbury, Mt Hutt, coll. R.J.McKenzie.

Helichrysum coralloides (Hook.f.) Benth. & Hook.f. × *H. depressum*. Canterbury, Mt Terako.

Helichrysum dimorphum × *Helichrysum filicaule*. **A**: Canterbury, seed ex Puffer's Stream, R.J.McKenzie 112/1-2.

Helichrysum filicaule.× *Raoulia glabra*. Banks Peninsula, Stony Bay Peak, R.J.McKenzie 293.

Helichrysum intermedium × *Helichrysum lanceolatum*. **A**: Canterbury, Ghost Creek, R.J.McKenzie 224/1-3.

Helichrysum intermedium var. *tumidum* × *Anaphalioides bellidioides* 'Graeme Paterson'. Otago Peninsula, seed ex Lovers' Leap, coll. G.Paterson.

Leucogenes grandiceps × *Raoulia eximia*. see p. 194–195.

Raoulia apicinigra × *Raoulia cinerea* Petrie. Marlborough, Balaclava Ridge, coll. A.D.Wilton.

Raoulia apicinigra × *Raoulia australis*. Marlborough, Barefell Pass, R.J.McKenzie 155.

Raoulia australis × *Raoulia parkii* Buchanan. Marlborough, Barefell Pass, R.J.McKenzie 165.

Raoulia australis × *Raoulia beauverdii*. Otago, Alexandra, The Lookout, J.M.Ward 94049/2.

Raoulia australis × *Raoulia subsericea*. Marlborough, Barefell Pass R.J.McKenzie 156.

Raoulia hectorii Hook.f. × *Raoulia subsericea*. **A**: Otago, The Remarkables, A.D.Wilton 309; **B**: Otago, Mt St Bathans, coll. J.M.Ward.

Appendix 3. Data sets for case study 1

The characters and character states used in the data analyses are listed below. The numerical code indicating the character type for calculating dissimilarities is presented in brackets: 1, continuous and ordered multistate; 2, unordered multistate. In the data matrices, the mean of up to ten measurements was used for each continuous character, and missing data and inapplicable characters were coded as -99.

3.1 Characters recorded

1. Growth form: 0 = mat-forming to sprawling; 1 = subshrubby to erect. (2)
2. Rooting pattern: 0 = taprooted; 1 = adventitious roots produced along entire stem. (2)
3. Nonflowering shoot orientation: 0 = prostrate or decumbent; 1 = erect. (2)
4. Morphologically distinct juvenile phase: 0 = absent; 1 = present. (2)
5. Distinct internodes on flowering shoots: 0 = absent; 1 = present. (2)
6. Leaf length (mm). (1)
7. Maximum lamina width (mm). (1)
8. Leaf length: maximum lamina width ratio. (1)
9. Point of maximum lamina width: leaf length ratio. (1)
10. Leaf / stem angle: 0 = appressed to stem; 1 = spreading. (2)
11. Leaf tip margins: 0 = plane; 1 = cucullate. (2)
12. Lamina margins: 0 = plane; 1 = upturned. (2)
13. Shape of lamina base: 0 = narrows abruptly; 1 = tapering. (2)
14. Stem enclosure by the leaf petiole extensions: 0 = ≤ 50% of stem enclosed; 1 = > 50 % of the stem enclosed. (2)
15. Mucro length (mm). (1)
16. Mucro orientation: 0 = recurved and pointing towards leaf axil (lamina/mucro angle < 90°); 1 = usually upturned (angle 90-180°); 2 = plane with leaf axis (angle ± 180°). (1)
17. Leaf indumentum density on adaxial lamina surface: 0 = dense; 1 = moderate; 2 = sparse to glabrous. (1)
18. Leaf indumentum density on abaxial lamina surface: 0 = dense; 1 = moderate; 2 = sparse to glabrous. (1)
19. Type B clothing trichomes on leaf: 0 = absent; 1 = present. (2)
20. Type B glandular trichomes on margins and adaxial surface of leaf: 0 = absent; 1 = present. (2)
21. Terminal cell shape of type A glandular trichomes: 0 = oval; 1 = oblong-oval; 2 = oblong. (1)
22. Terminal cell length of type A glandular trichomes: 0 = 12-18 μm; 1 = 14-23 μm; 2 = 19-27 μm; 3 = 28-35 μm. (1)
23. Density of type B glandular trichomes on adaxial lamina surface and leaf margins: 0 =common; 1 = sparse or rare; 2 = absent. (2)
24. Number of basal cells in clothing trichomes: 0 = always one cell; 1 = one or two cells. (2)
25. Base of terminal cell of clothing trichomes: 0 = swollen; 1 = not swollen. (2)
26. Nerves raised on adaxial lamina surface: 0 = absent; 1 = present. (2)
27. Midrib raised on abaxial leaf surface: 0 = absent; 1 = present. (2)
28. Lateral nerves raised on abaxial leaf surface: 0 = absent; 1 = present. (2)
29. Cuticle thickness: 0 = thicker on adaxial lamina surface; 1 = equal thickness on both lamina surfaces. (2)

30. Epidermis thickness: 0 = thicker on adaxial lamina surface; 1 = equal thickness on both lamina surfaces. (2)
31. Stomata level: 0 = level with epidermis; 1 = guard cells raised; 2 = guard cells and adjacent cells raised. (1)
32. Palisade chlorenchyma in midrib: 0 = absent; 1 = present. (2)
33. Abaxial collenchyma in midrib and lateral ribs: 0 = absent; 1 = present. (2)
34. Spongy mesophyll differentiation: 0 = well differentiated; 1 = slightly differentiated. (2)
35. Morphologically distinct flowering shoots: 0 = absent; 1 = present. (2)
36. Transition from leaves to involucral bracts: 0 = abrupt; 1 = gradual. (2)
37. Capitulum pedunculate: 0 = absent; 1 = present. (2)
38. Number of capitula per inflorescence. (1)
39. Capitulum length (mm). (1)
40. Capitulum width at midpoint (mm). (1)
41. Number of female florets per capitulum. (1)
42. Number of hermaphrodite florets per capitulum. (1)
43. Total number of florets per capitulum. (1)
44. Female: total floret ratio. (1)
45. Receptacle height (mm). (1)
46. Receptacle diameter (mm). (1)
47. Receptacle type: 0 = alveolate, foveolate or fimbrillate; 1 = scrobiculate. (2)
48. Receptacle scales: 0 = absent; 1 = present. (2)
49. Inner involucral bract length (mm). (1)
50. Inner involucral bract, lamina length (mm). (1)
51. Inner involucral bract, maximum width (mm). (1)
52. Colour of lamina of inner involucral bracts: 0 = white; 1 = pale yellow. (2)
53. Lamina of inner involucral bracts hygroscopic: 0 = absent; 1 = present. (2)
54. Shape of lamina tip of inner involucral bracts: 0 = acute to obtuse; 1 = obtuse to rounded; 2 = rounded. (1)
55. Reddish coloration in lamina/stereome gap of outer involucral bract: 0 = absent; 1 = present. (2)
56. Hyaline margins on stereome of inner involucral bract: 0 = absent; 1 = present. (2)
57. Corolla tube length in female florets (mm). (1)
58. Corolla tube length in hermaphrodite florets (mm). (1)
59. Upper corolla tube crimson at anthesis: 0 = absent; 1 = present. (2)
60. Corolla tube of outermost florets strongly curved: 0 = absent; 1= present. (2)
61. Corolla lobe colour at anthesis: 0 = green; 1 = greenish-white; 2 = white; 3 = yellow. (2; 1 when state 3 excluded)
62. Corolla lobes develop crimson coloration with age: 0 = absent; 1 = present. (2)
63. Corolla lobes recurved: 0 = erect only; 1 = at least some lobes partly patent or recurved. (2)
64. Crimson coloration in anthers: 0 = dark crimson; 1 = pale reddish; 2 = absent. (1)
65. Pollen colour: 0 = yellow; 1 = pale yellow; 2 = white. (1)
66. Style arm colour: 0 = white; 1 = pale green to greenish-white; 2 = yellow. (2)
67. Pappus hair length in female florets (mm). m(1)
68. Female floret pappus hairs, number of apical cells: 0 = one or two; 1 = one to three; 2 = three to six. (1) Also defined as: 0 = one or two; 1 = one to five. (2)
69. Female floret pappus hairs distinctly dimorphic: 0 = absent; 1 = present. (2)
70. Female floret pappus hairs, apical cells distinctly protruding: 0 = absent; 1 = present. (2)
71. Pappus hair length in hermaphrodite florets (mm). (1)
72. Hermaphrodite floret pappus hairs, number of apical cells: 0 = one or two; 1 = one to four; 2 = three to five; 3 = five to eight. (1)
73. Pappus hairs, shape of apical cells: 0 = acute; 1 = clavate. (2)

74. Type of wall thickening in pappus hair apical cells: 0 = reticulate or irregular; 1 = uniformly thickened. (2)
75. Density of basal cilia on pappus hairs: 0 = sparse; 1 = moderate to dense. (2)
76. Length of basal cilia on pappus hairs: 0 = up to 12 μm long; 1 = up to 25 μm long; 2 = maximum > 25 μm long. (1)
77. Angle of basal cilia on pappus hairs: 0 = ascending only; 1 = ascending, spreading or recurved. (2)
78. Female floret ovary length (mm). (1)
79. Female floret ovary width (mm). (1)
80. Female floret ovary length: width ratio. (1)
81. Hermaphrodite floret ovary length (mm). (1)
82. Hermaphrodite floret ovary width (mm). (1)
83. Hermaphrodite floret ovary length:width ratio. (1)
84. Ovary epidermal cell shape: 0 = rounded; 1 = smooth. (2)
85. Twin hairs on ovary of female florets: 0 = absent; 1 = present. (2)
86. Twin hairs on ovary of hermaphrodite florets: 0 = absent; 1 = present. (2)
87. Twin hairs, shape of terminal cells: 0 = acute; 1 = clavate. (2)
88. Multicellular twin hairs on ovary of female florets: 0 = absent; 1 = present. (2)
89. Multicellular twin hairs on ovary of hermaphrodite florets: 0 = absent; 1 = present. (2)

3.2 Characters included in each analysis

Key: c, characters recorded from cultivated plants; f, characters recorded from field-collected specimens. †, log transformed; ‡, square-root transformed.

Character	Putative hybrids and all sympatric Gnaphalieae included		Putative hybrids and only putative parental species included		
	HYWIN, MDS, split decomposition	MDA	Character count	Character index	Cluster, HYWIN, MDS, split decomposition
1	f		c	c f	c f
2	f		c	c f	c f
3	f		c	c f	c f
4	f				
5	f				
6	f	f	c f	c f	c f
7		f	c	c f	c f
8	f	f	c f		
9	f	f ‡			
10	f				
11	f				
12	f				
13	f				
14	f		c	c f	c f
15	f	f †	c f	c f	c f
16	f		c	c f	c f
17	f		c	c f	c f
18	f				
19	f				
20	f		c	c f	
21			c	c f	c f
22			c	c f	c f
23					c f
24			c	c f	c f
25					
26	f		c		
27	f				
28	f			c f	c f
29			c	c	c
30			c	c	c
31			c	c	c
32			c	c	c
33			c	c	c
34			c	c	c
35	f				
36	f				
37	f				

3.2 Characters included in each analysis (continued)

Character	Putative hybrids and all sympatric Gnaphalieae included		Putative hybrids and only putative parental species included		
	HYWIN, MDS, split decomposition	MDA	Character count	Character index	Cluster, HYWIN, MDS, split decomposition
38	f	f ‡	c f	c f	c f
39	f	f †	c f	c f	c f
40	f	f †	c f	c f	c f
41	f	f ‡	c f	c f	c f
42	f	f ‡	c f	c f	c f
43		f ‡	c f		
44			c f		
45	f	f †	c f	c f	c f
46	f	f	c f	c f	c f
47	f		c	c f	c f
48	f				
49	f	f †	c f	c f	c f
50			c f	c f	c f
51	f	f			
52	f				
53	f				
54	f		c	c f	c f
55	f		c	c f	c f
56			c	c f	c f
57	f		c f	c f	c f
58	f	f †	c f	c f	c f
59	f		c	c f	c f
60	f				
61	f		c	c f	c f
62	f		c	c f	c f
63	f		c	c f	c f
64	f			c f	c f
65	f		c	c f	c f
66			c		
67	f		c f	c f	c f
68	f		c	c f	c f
69			c	c	f
70	f		c	c f	c f
71	f	f	c f	c f	c f
72	f		c	c f	c f
73	f				
74	f		c	c f	c f

3.2 Characters included in each analysis (continued)

Character	Putative hybrids and all sympatric Gnaphalieae included		Putative hybrids and only putative parental species included		
	HYWIN, MDS, split decomposition	MDA	Character count	Character index	Cluster, HYWIN, MDS, split decomposition
75			c	c f	f
76			c	c f	f
77			c	c f	f
78	f				
79					
80	f				
81	f	f †			
82		f †			
83	f	f			
84			c	c f	c f
85	f		c	c f	c f
86	f		c	c	c
87	f				
88	f		c	c	c
89	f				

3.3 Matrix of characters recorded from field-collected specimens

Character	*A. bellidioides*						*E. sinclairii*									*H. coralloides*			*H. parvifolium*			*O. leptophyllus*					Putative hybrids					
	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	1	2	3	1	2	3	1	2	3	4	5	*W4*	*W5*	*W8*	*W9*	*W10*	*W12*
1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	-99	-99	-99	0	0	1
2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	-99	-99	-99	2	2	1
3	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-99	-99	-99	0	0	-99
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	-99	-99	-99	0	0	-99
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
6	4.95	4.1	5.39	4.43	6.56	5.29	7.91	7.97	9.21	11.4	13.1	11	11.2	13.2	14	5.77	4.89	5.33	1.54	1.59	1.64	7.35	8.26	7.82	7.15	7.86	5.1	-99	5.62	7.57	6.17	3.81
7	2.06	2.05	2.43	2.04	2.59	2.25	2.14	1.89	2.3	2.66	3.76	3.15	2.72	3.03	3.2	3.43	2.87	3.33	1.1	1.08	1.08	2.59	2.92	2.86	2.16	2.67	1.99	-99	2.26	3.15	2.25	1.63
8	2.41	1.98	2.23	2.18	2.53	2.36	3.7	4.24	4	4.34	3.5	3.51	4.13	4.36	4.39	1.69	1.71	1.61	1.4	1.48	1.52	2.84	2.84	2.77	3.32	2.95	2.57	-99	2.49	2.4	2.75	2.33
9	0.31	0.3	0.3	0.32	0.32	0.26	0.28	0.25	0.36	0.3	0.34	0.36	0.36	0.27	0.31	0.67	0.75	0.83	0.9	0.91	0.9	0.37	0.35	0.33	0.3	0.32	0.26	-99	0.29	0.27	0.29	0.36
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	-99
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	-99
13	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0
14	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-99	1	1	1	1
15	0.24	0.22	0.27	0.25	0.32	0.25	0.09	0.11	0.09	0.09	0.14	0.11	0.1	0.12	0.18	0	0	0	0.04	0.04	0.04	0.06	0.13	0	0.01	0.01	0.34	-99	0.2	0.19	0.24	0.19
16	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	-99	-99	-99	-99	-99	-99	2	2	-99	2	2	1	-99	1	1	2	1
17	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	1	-99	1	1	1	1
18	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	2	2	2	2	2	2	-99	2	2	2	2
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	-99	0	0	0	0
20	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-99	0	1	1	1
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	-99	0	0	0	0
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	-99	1	1	1	1
28	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	-99	0	1	1	1
35	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
38	1	1	1	1	1	1	8	25	12.7	24	12	24	24.7	19.4	27.2	1	1	1	1	1	1	48.7	28.9	34.9	18.5	29.1	4	5	4	3.88	1.86	1
39	6.55	6.63	7.68	7.64	7.7	7.69	4.81	4.71	4.82	4.97	4.53	4.95	4.73	5.06	5.36	6.74	7.13	7.58	5.38	5.84	5.56	4.24	4.99	4.55	4.71	4.65	5.7	6.06	6.12	6.12	6.33	-99
40	6.4	7.08	5.93	6.81	7.53	6.4	2.36	2.58	2.68	2.65	2.49	2.6	2.73	2.54	2.65	4.11	3.88	4.63	2.72	2.93	2.67	2.16	2.54	2.3	2.06	2.04	3.9	4.68	3.84	3.74	4.21	-99
41	84.5	98.3	82.3	94.7	106	97.9	9.4	9	9.5	9.2	7.86	10	10	9.2	9.8	11.2	23.9	22	5.7	10.3	7.6	0	0	0	0	0	39	37	40	36.5	36	22
42	80	94	70.8	87.6	91.8	87.9	15.2	17	18.8	19.7	13.9	17.3	21.3	17.6	17	46.2	62.7	64.4	30.1	27.4	23.3	17.2	12.5	11.2	9.4	9.7	39	43	42	30.9	47.4	28
43	165	192	153	182	198	186	24.6	26	28.3	28.9	21.7	27.3	31.3	26.8	26.8	57.4	86.6	86.4	35.8	37.7	30.9	17.2	12.5	11.2	9.4	9.7	78	80	82	67.4	83.4	50
44	0.51	0.51	0.54	0.52	0.53	0.53	0.38	0.34	0.34	0.32	0.36	0.37	0.32	0.35	0.37	0.2	0.28	0.26	0.16	0.28	0.25	-99	-99	-99	-99	-99	0.5	0.46	0.49	0.55	0.43	0.44
45	2.04	2.06	2.12	2.22	2.41	2.69	0.19	0.08	0.27	0.19	0	0.21	0.01	0	0.17	0.49	0.42	0.69	0.32	0.34	0.26	0.38	0.44	0.27	0.21	0.26	0.65	0.5	0.73	0.74	0.94	1.2
46	2.22	2.22	2.22	2.44	2.39	2.3	1.44	1.49	1.45	1.52	1.5	1.51	1.6	1.48	1.59	1.56	2.32	2.47	1.45	1.42	1.22	0.97	0.93	0.71	0.64	0.62	1.63	1.75	1.75	1.63	1.63	1.9
47	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0

3.3 Characters recorded from field-collected specimens (continued)

Character	*A. bellidioides*						*E. sinclairii*									*H. coralloides*			*H. parvifolium*			*O. leptophyllus*					Putative hybrids					
	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	1	2	3	1	2	3	1	2	3	4	5	*W4*	*W5*	*W8*	*W9*	*W10*	*W12*
49	7.69	7.79	8.6	8.59	8.9	8.2	4.17	4.14	4.57	4.28	3.9	4.28	3.62	4.07	4.39	6.04	5.16	5.96	3.86	4.27	3.95	3.51	4.23	3.5	4.18	3.94	6.01	6.04	5.99	6.67	6	5.32
50	4.73	4.66	5.4	5.35	5.78	5.31	1.83	1.9	1.75	2	1.78	2.01	1.67	2.04	2.15	-99	-99	-99	-99	-99	-99	1.04	1.1	0.77	0.96	1.03	3.38	3.24	3.38	3.93	3.45	3.37
51	1.18	1.6	1.36	1.21	1.44	1.33	1.29	1.17	1.29	1.28	1.11	1.24	1.16	1.11	1.24	1.45	1.28	1.23	1.23	1.44	1.18	1.2	1.3	0.94	1.06	1.12	0.84	1.38	1.19	1.19	1.02	0.87
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
53	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
54	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	2	2	2	2	2	1	1	1	1	0	0
55	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	-99	-99	-99	0	0	1
57	2.24	2.38	2.65	2.6	2.52	2.39	2.02	2.03	1.89	1.92	2.13	2.13	2.04	2.4	2.36	3.43	3.44	3.76	2.82	3.05	2.52	-99	-99	-99	-99	-99	2.2	2.42	2.22	2.39	2.33	2.65
58	2.37	2.66	2.72	2.68	2.57	2.59	2.29	2.28	2.13	2.2	2.33	2.28	2.37	2.66	2.48	3.39	4.04	3.9	3.1	3.25	3.12	2.2	2.69	2.38	2.54	2.59	2.62	2.72	2.58	2.77	2.55	2.94
59	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	-99	-99	-99	0	0	-99
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
61	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	2	2	2	2	2	-99	-99	-99	1	1	-99
62	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-99	-99	-99	0	1	1
63	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
64	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	-99	-99	-99	1	1	-99
65	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	1	1	1	1	1	-99	-99	-99	1	1	-99
66	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	0	0	0	0	0	-99	-99	-99	1	1	-99
67	2.91	3.26	3.28	3.53	3.6	3.08	2.44	2.44	2.22	2.33	2.52	2.5	2.49	2.72	2.7	3.75	3.5	3.81	2.72	3.27	2.96	-99	-99	-99	-99	-99	2.79	3.33	2.83	2.97	3.27	3.57
68	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	-99	-99	-99	-99	-99	1	1	1	1	1	1
70	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	-99	-99	-99	-99	-99	0	0	0	0	0	1
71	3.26	3.42	3.42	3.79	3.74	3.48	2.71	2.64	2.4	2.5	2.63	2.58	2.66	2.82	2.85	3.95	3.95	3.99	2.81	3.45	2.99	2.88	3.13	2.68	2.89	3.06	2.99	3.56	3.16	3.23	3.37	3.55
72	0	0	0	0	0	0	3	3	3	3	3	3	3	3	3	2	2	2	1	1	1	2	1	1	2	2	2	2	2	2	2	2
73	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
74	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
78	0.65	0.74	0.66	0.78	0.75	0.66	0.81	0.7	0.65	0.75	0.74	0.77	0.89	0.87	0.75	0.93	1.3	1.35	1.07	1.21	1.06	-99	-99	-99	-99	-99	0.58	0.69	0.6	0.61	0.5	0.89
79	0.21	0.25	0.22	0.23	0.45	0.24	0.3	0.32	0.33	0.31	0.35	0.32	0.34	0.32	0.33	0.31	0.31	0.33	0.31	0.33	0.35	-99	-99	-99	-99	-99	0.18	0.19	0.17	0.25	0.26	0.26
80	0.32	0.34	0.33	0.29	0.6	0.36	0.37	0.45	0.51	0.42	0.48	0.41	0.38	0.36	0.43	0.33	0.24	0.24	0.29	0.27	0.33	-99	-99	-99	-99	-99	0.31	0.27	0.29	0.42	0.51	0.29
81	0.69	0.73	0.69	0.8	0.74	0.65	0.76	0.75	0.65	0.74	0.83	0.8	0.86	0.89	0.75	1.01	1.29	1.16	1.11	1.19	1.03	0.77	0.93	0.87	0.86	0.8	0.57	0.64	0.56	0.66	0.76	0.93
82	0.28	0.31	0.27	0.3	0.28	0.29	0.34	0.33	0.35	0.32	0.31	0.34	0.35	0.35	0.32	0.36	0.36	0.32	0.31	0.34	0.35	0.38	0.4	0.43	0.45	0.38	0.2	0.2	0.19	0.28	0.27	0.29
83	0.4	0.42	0.39	0.37	0.38	0.45	0.45	0.44	0.53	0.43	0.37	0.43	0.41	0.39	0.43	0.36	0.28	0.28	0.28	0.29	0.34	0.49	0.43	0.5	0.52	0.48	0.35	0.31	0.34	0.42	0.35	0.31
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	-99	-99	-99	-99	-99	0	1	0	0	0	1
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
87	-99	-99	-99	-99	-99	-99	-99	-99	-99	-99	-99	-99	-99	-99	-99	1	1	1	0	0	0	-99	-99	-99	-99	-99	-99	1	-99	-99	-99	1
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-99	-99	-99	-99	-99	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0

3.4 Matrix of characters recorded from cultivated plants

Character	*A. bellidioides*						*E. sinclairii*					Putative hybrids								
	1	2	3	4	5	6	1	2	3	4	5	*W1*	*W2*	*W9*	*W10*	*W11*	*W13*	*S1*	*S2*	*S3*
1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
2	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
3	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
6	14.43	14.19	14.03	13.72	13.34	14.05	18.81	19.11	19.53	21.36	21.03	16.15	18.69	17.14	14.12	18.89	18.94	15.54	14.58	14.66
7	5.69	5.6	5.55	5.44	5.33	5.68	6.48	6.19	5.67	6.67	6.6	5.01	6.21	6.15	4.82	6.94	6.19	5.76	5.19	5.29
8	2.53	2.55	2.53	2.53	2.51	2.48	2.91	3.09	3.48	3.21	3.19	3.21	3	2.79	2.94	2.73	3.06	2.48	2.79	2.78
14	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
15	0.39	0.394	0.39	0.389	0.404	0.38	0.174	0.165	0.178	0.17	0.176	0.298	0.323	0.443	0.348	0.435	0.27	0.245	0.423	0.395
16	2	2	2	2	2	2	0	0	0	0	0	2	2	2	2	2	1	1	2	1
17	2	2	2	2	2	2	0	0	0	0	0	1	1	1	1	1	1	1	2	2
20	0	0	0	0	0	0	2	2	2	2	2	1	1	1	1	1	1	1	1	1
21	2	2	2	2	2	2	0	0	0	0	0	1	1	1	1	1	1	1	1	1
22	0	0	0	0	0	0	3	3	3	3	3	1	1	1	1	1	1	1	0	2
24	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	0	0	1
25	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1
29	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	2	2	2	2	2	1	1	1	1	1	1	1	0	0
32	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
33	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
38	1	1	1	1	1	1	31.7	48	16	22	53.9	2.8	3.3	3.3	3.1	2.9	2.5	2.9	1	1
39	8.47	9.9	9.63	9.25	9.42	9.56	4.61	4.42	4.37	4.53	4.88	6.57	6.73	6.56	7.11	6.91	6.89	6.95	6.8	6.59
40	6.61	6.41	6.61	5.91	6.69	6.47	2.31	2.57	2.11	2.3	2.2	4.37	4.7	3.36	3.52	3.82	4.46	4.53	4.44	3.91
41	118.5	126.5	127.1	136.2	138.2	127.5	12.3	13.9	-99	12.2	10.4	40.9	42.7	40.9	41.9	54.4	37.5	47.1	75.2	82.1
42	95.2	110.5	114.8	122.5	133.5	115	27.6	29	-99	21.9	21.5	47.7	50.1	45.9	49.2	45.3	44.5	57.5	73.9	52.9
43	212.8	237	241.9	258.7	271.7	242.5	39.9	42.9	-99	34.1	31.9	88.6	92.8	86.8	91.1	98.7	82	104.6	148.1	135
44	0.551	0.536	0.528	0.527	0.507	0.525	0.307	0.324	-99	0.357	0.327	0.462	0.461	0.474	0.459	0.551	0.456	0.452	0.509	0.608
45	2.33	2.32	2.2	2.17	2.5	2.43	0	0.35	0	0.3	0.38	0.73	0.87	1.12	1.19	1.02	0.67	1.29	1.48	1.43
46	2.54	2.9	2.79	2.74	2.89	2.91	1.35	1.3	-99	1.29	1.31	1.98	1.89	1.74	1.805	2.01	2.104	2.196	2.403	1.869
47	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1
49	9.24	8.96	9.08	8.49	8.62	8.92	3.82	3.67	4.05	3.78	3.77	6	6.93	6.49	6.07	6.13	6.38	7.17	6.65	6.68
50	6.24	5.9	6.07	5.95	5.78	6.11	1.72	1.59	1.64	2.44	2.09	3.86	4.44	4.3	3.95	3.26	4.14	4.21	3.79	4.22
54	0	0	0	0	0	0	2	2	2	2	2	1	1	1	0	1	1	0	0	1
55	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	1	1	1

3.4 Characters recorded from cultivated plants (continued)

Character	*A. bellidioides*						*E. sinclairii*					Putative hybrids								
	1	2	3	4	5	6	1	2	3	4	5	*W1*	*W2*	*W9*	*W10*	*W11*	*W13*	*S1*	*S2*	*S3*
56	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1
57	2.89	2.75	2.29	2.65	2.65	2.85	1.78	1.78	1.92	1.85	1.84	2.29	2.4	2.52	2.3	2.51	2.5	2.48	2.28	2.17
58	3.15	3.08	4.35	4.34	3.0	3.2	1.91	1.94	2.14	2.06	2.13	2.79	2.76	3.0	2.66	2.98	2.73	2.67	2.64	2.59
59	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	2	2	2	2	2	1	1	1	1	1	1	0	0	0
62	1	1	0	1	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1
63	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1
64	2	2	2	2	2	2	0	0	0	0	0	1	1	1	1	1	1	2	2	2
65	0	0	0	0	0	0	2	2	2	2	2	1	1	1	1	1	2	0	0	0
67	3.71	3.53	3.21	3.33	3.51	3.46	1.95	2.01	2.24	2.16	2.26	3.37	3.45	3.19	3.28	3.14	3.4	3.6	3.26	2.73
68	0	0	0	0	0	0	1	1	-99	1	1	1	1	1	1	1	1	1	1	1
69	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	0	0	0
71	3.77	3.76	3.71	3.37	3.84	3.75	2.02	2.02	2.45	2.39	2.60	3.08	3.52	3.31	3.39	3.19	3.45	3.89	3.65	3.32
72	0	0	0	0	0	0	2	2	-99	2	2	1	1	1	1	1	1	1	1	1
74	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0
76	0	0	0	0	0	0	1	1	2	1	1	1	1	1	1	2	2	0	1	2
77	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
84	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
85	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Appendix 4. Fluorochromatic reaction (FCR) tests

The performance of fluorescein diacetate as an indicator of pollen fertility was tested on plants of *Anaphalioides bellidioides*, *A. trinervis* and a putative *Anaphalioides bellidioides* × *Ewartia sinclairii* hybrid (*F11*). The FCR reaction indicates membrane integrity and esterase activity in intact grains (Dafni & Firmage, 2000). The method followed Heslop-Harrison *et al.* (1984). Only freshly presented pollen was used. A range of sucrose concentrations was evaluated to minimise pollen bursting due to an osmotic imbalance. In three tests fluorescence was monitored at intervals over a 3–4 h period (and again after 24 h in two tests). At all sucrose concentrations fluorescence of the cytoplasm was usually weak to moderate; only in occasional grains was strong fluorescence observed.

5.1 *Anaphalioides trinervis* pollen

Freshly presented pollen grains from *A. trinervis D.Glenny 4585* (a plant collected from the Aorere Valley, Nelson) were placed in a range of sucrose concentrations (0–2.8 M) containing fluoroscein diacetate. The presence of fluorescence was scored for 200 pollen grains at intervals over a 4 h period.

Fluorescence developed within 5 min in the water and 1 M sucrose solutions (Fig. X.1), but the majority of grains were non-fluorescing or had burst within 5 min in water. In the 1 M solution, 43.5 % of the grains fluoresced after 5 min; the proportion of fluorescent grains began to decline after 40 min and after 4 h only 10 % of the grains fluoresced weakly. Fluorescence was slower to develop but was emitted over a longer period at the higher sucrose concentrations. In the 1.5 M sucrose solution, 59.5 % of the grains fluoresced after 10 min. The level began to decline after 2.5 h and fell to 29 % after 4 h. In the 2 M sucrose solution, 64 % of the grains fluoresced weakly after 20 min and fluorescence intensity increased with time. The proportion of fluorescing grains began to decline after 90 min but the rate of decline was slower than for the 1.5 M solution, and 40.5 % of the grains still fluoresced moderately to strongly after 4 h. In the 2.8 M solution only a single grain fluoresced moderately after 90 min; fluorescence in all other grains was absent or extremely faint.

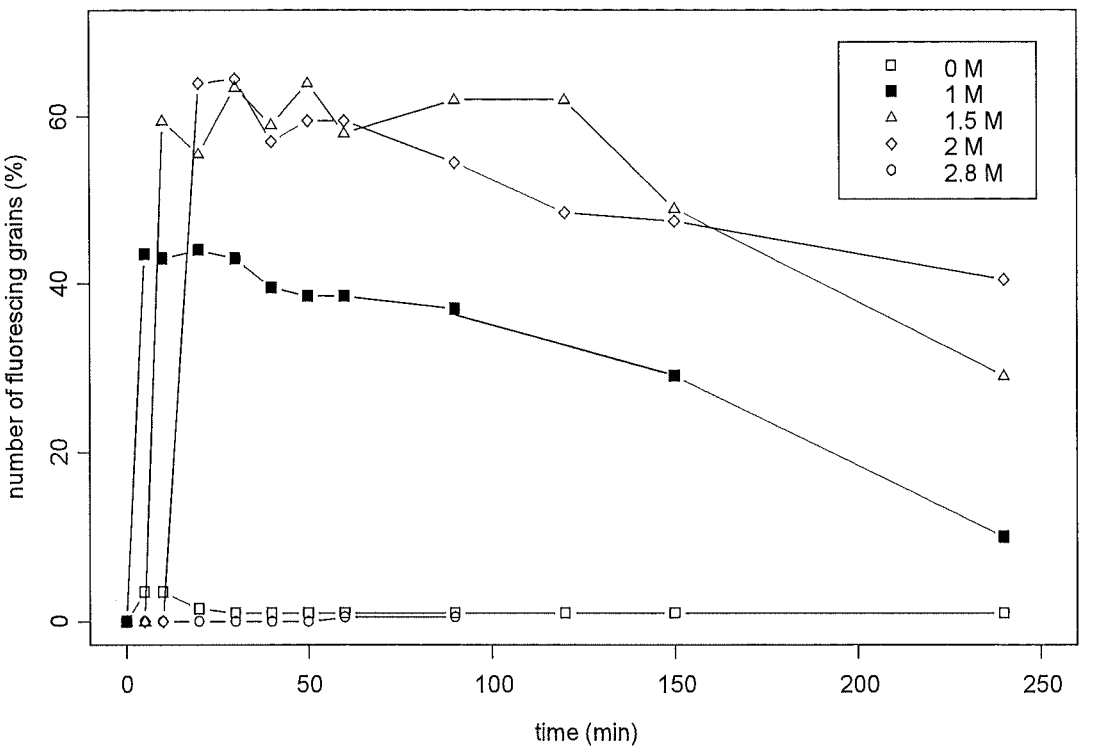

Figure 1. Proportion of fluorescing pollen grains of *A. trinervis D.Glenny 4585* with time after placement in a range of sucrose solutions (0 M – 2.8 M) containing fluorescein diacetate. 200 pollen grains were counted for each point.

5.2 *Anaphalioides trinervis* pollen in 2 M sucrose

Freshly presented pollen grains were placed in a 2 M sucrose solution containing fluoroscein diacetate. The presence of fluorescence was scored for 200 grains on five replicate slides at intervals over a 3 h period and again after 24h.

No fluorescing grains were observed after 15 min (Fig. X.2). After 30 min 27.7 % of the grains fluoresced , peaking at 71.8 % after 90 min. The proportion of fluorescing grains declined to 62.2 % after 3 h. After 24 h, 48.1 % of the grains still fluoresced.

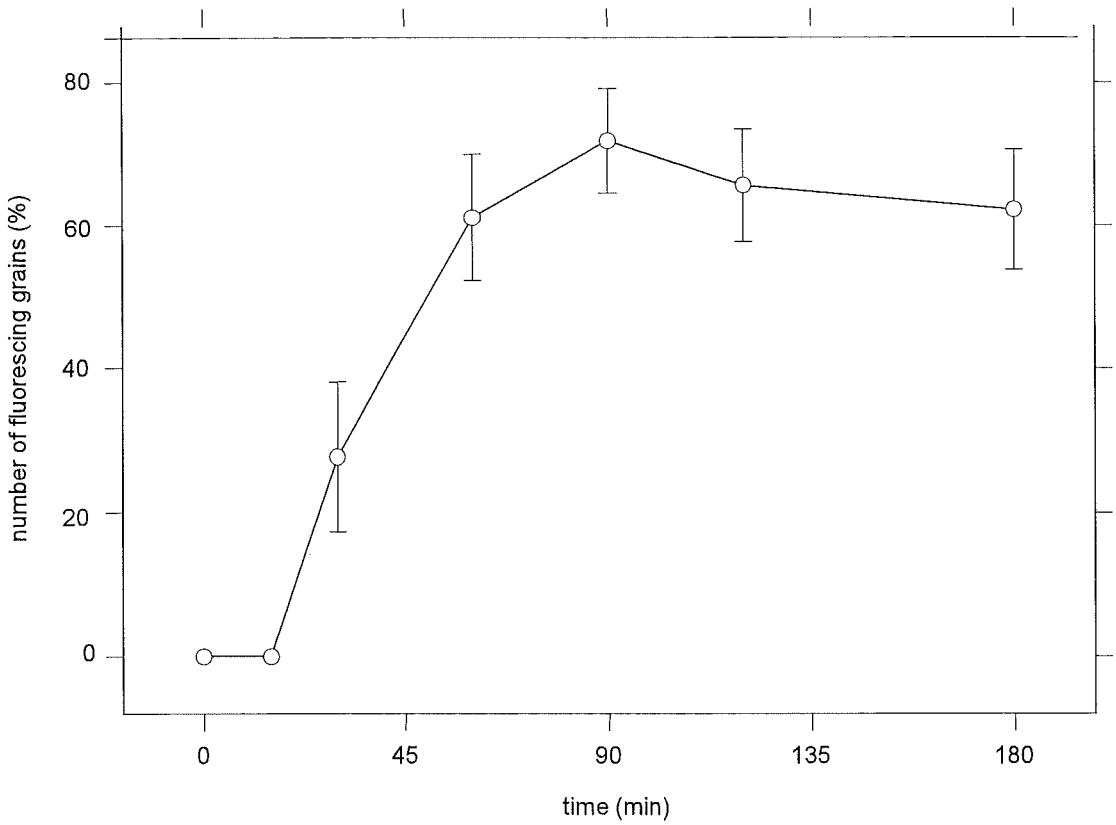

Figure 2. Proportion of fluorescing pollen grains of *A. trinervis D.Glenny 4585* with time after placement in a 2 M sucrose solution containing fluorescein diacetate. Points are the mean ± s.d. for five replicate slides, with 200 pollen grains counted per slide.

5.3 *Anaphalioides bellidioides* pollen

Freshly presented pollen grains from *A. bellidioides RMcK 130* (a plant collected from Ashley Gorge, Canterbury) were placed in a range of sucrose concentrations (0–3 M) containing fluoroscein diacetate. The number of fluorescing grains were counted after 1 h.

At sucrose concentrations below 2 M, fluorescence developed more rapidly, but the frequency of non-fluorescing grains was also higher and the proportion of burst pollen grains increased with time. At higher sucrose concnetrations (2 M and above), fluorescence was slower to develop (after a minimum of 30 min), but non-fluorescing grains were much less frequent. The 2.5 M sucrose solution possessed the highest mean proportion of fluorescing grains (95.3 %).

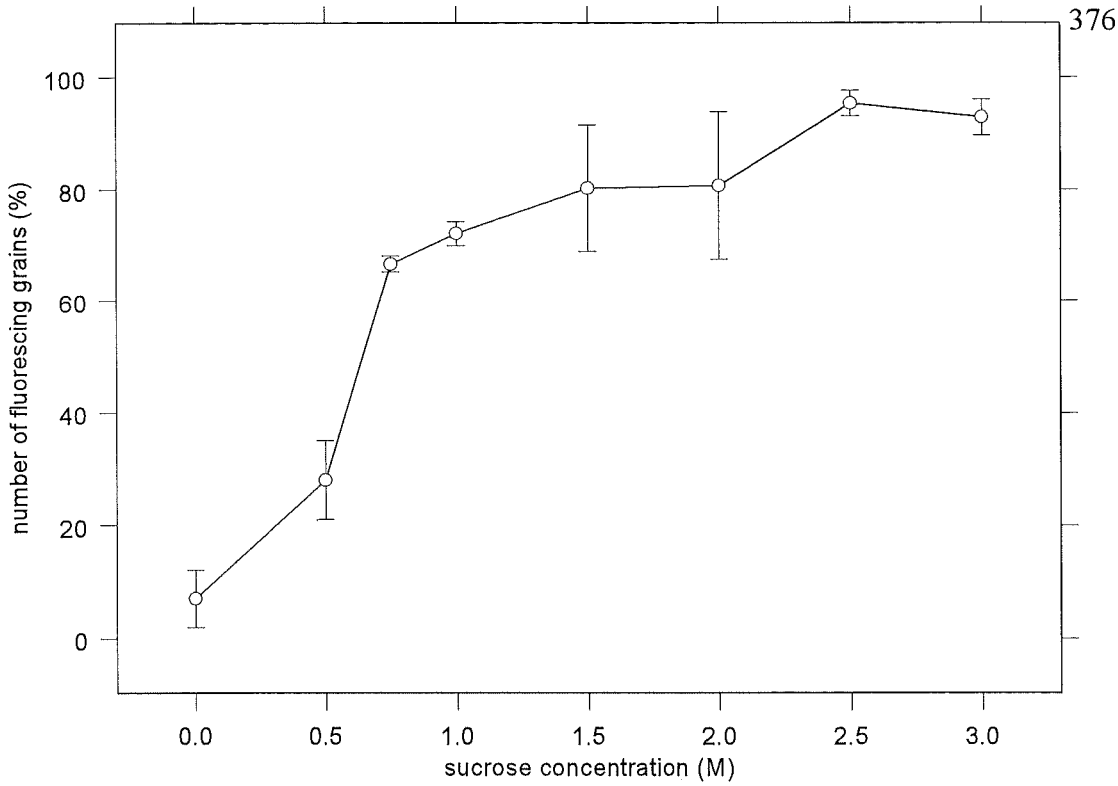

Figure 3. Proportion of fluorescing pollen grains of *A. bellidioides RMcK 130* scored 1 h after placement in sucrose solutions containing fluorescein diacetate. Points are the mean ± s.d. for three replicate slides, with 200 pollen grains counted per slide.

5.4 Putative *Anaphalioides bellidioides* × *Ewartia sinclairii* hybrid (*F11*)

Freshly presented pollen grains from *F11* were placed in a range of sucrose concentrations (1–2.8 M) containing fluoroscein diacetate. The level of fluorescence was scored for 200 grains at intervals over a 3 h period and again after 24 h.

Fluorescing grains were only observed after 10 min. Fluorescence was slower to develop and increased in intensity with time at the higher sucrose concentrations. The proportion of fluorescing grains never exceeded 1.5 % in the 1 M sucrose solution and in the 2 M solution peaked at 16 % after 3 h. The highest proportion of fluorescing grains (35 %) was recorded in the 2.5 M solution after 2 h. The percentage of fluorescing grains peaked at 28 % after 1 h in the 2.8 M solution and remained constant after 3 h. After 24 h, 10 % of the grains still fluoresced in 2 M sucrose, increasing to 15.5 % in the 2.8 M solution.

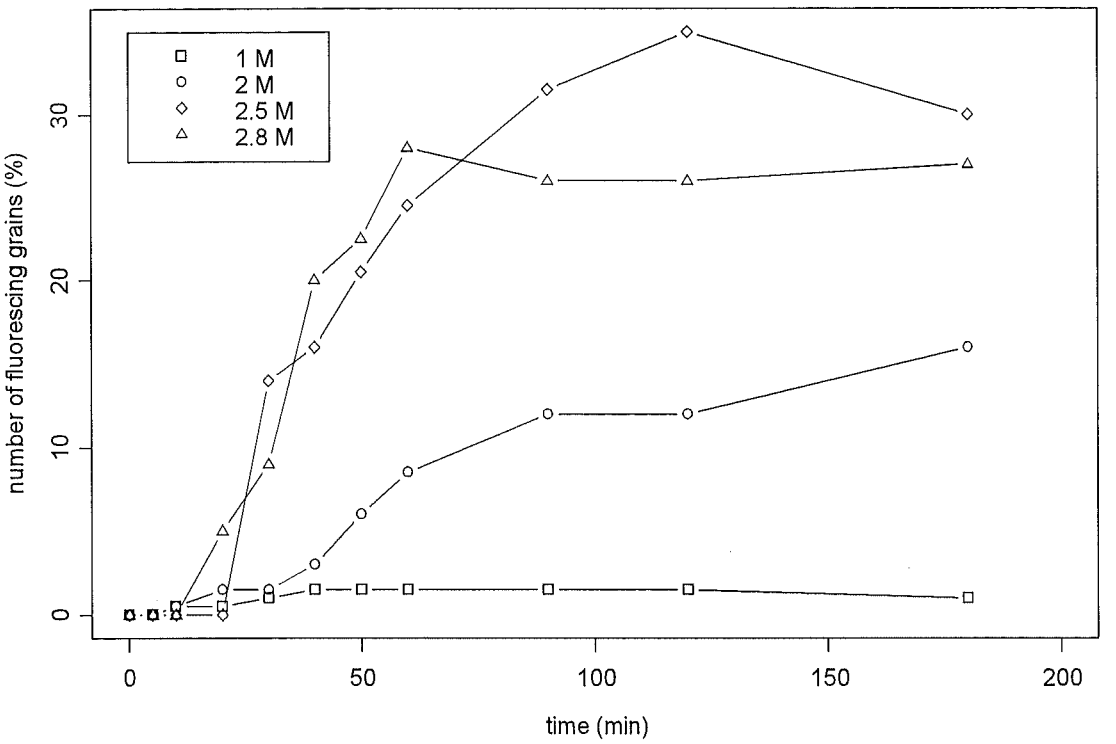

Figure 4. Proportion of fluorescing pollen grains of a putative *Anaphalioides bellidioides* × *Ewartia sinclairii* hybrid (*F11*) with time after placement in a range of sucrose solutions (1 M –2.8 M) containing fluorescein diacetate. 200 pollen grains were counted for each point.

References

Dafni, A., and Firmage, D. 2000. Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant Systematics and Evolution* 222: 113-132.

Heslop-Harrison, J., Heslop-Harrison, Y., and Shivanna, K. R. 1984. The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical and Applied Genetics* 67: 367-375.

Appendix 5. Data sets for case study 2

The characters and character states used in the data analyses are listed below. The numerical code indicating the character type for calculating dissimilarities is presented in brackets. In the data matrices, the mean of up to ten measurements was used for each continuous character, and missing data and inapplicable characters were coded as -99.

5.1. Characters recorded

1. Growth form: 0 = mat; 1 = mat or subshrub; 2 = cushion. (1)
2. Nonflowering shoot orientation: 0 = prostrate; 1 = prostrate to erect. (2)
3. Distinct internodes on both non-flowering and flowering shoots: 0 = absent; 1 = present. (2)
4. Leaf length (mm). (1)
5. Maximum lamina width (mm). (1)
6. Leaf length: maximum lamina width ratio. (1)
7. Leaf shape: 0 = strongly obovate; 1 = lanceolate; 2 = oblong to weakly obovate; 3 = linear. (2)
8. Leaf apex shape: 0 = acute; 1 = obtuse; 2 = rounded. (1)
9. Stem enclosure by the leaf petiole extensions: 0 = ≤ 50% of stem enclosed; 1 = > 50 % of the stem enclosed. (2)
10. Mucro length (mm). (1)
11. Mucro orientation: 0 = usually upturned (angle 90-180°); 1 = plane with leaf axis (angle ± 180°). (1)
12. Indumentum density on adaxial lamina surface: 0 = dense; 1 = moderate; 2 = sparse to glabrous. (1)
13. Type A clothing trichomes on leaf: 0 = absent; 1 = present. (2)
14. Type B clothing trichomes on leaf: 0 = absent; 1 = present. (2)
15. Clothing trichome terminal cell appressed on adaxial lamina surface: 0 = all ± appressed to leaf surface and interwoven; 1 = some appressed and interwoven, some not appressed and interwoven; 2 = not appressed and interwoven. (1)
16. Clothing trichome terminal cells on abaxial leaf surface: 0 = all ± appressed to leaf surface and interwoven; 1 = some appressed, some not appressed and interwoven; 2 = not appressed and interwoven. (1)
17. Number of basal cells in clothing trichomes: 0 = predominantly one; 1 = predominantly two or three. (2)
18. Clothing trichome basal cell length (mm). (1)
19. Clothing trichome basal cell width (mm). (1)
20. Clothing trichome terminal cell width (mm). (1)
21. Leaf glandular trichomes, number of cell series: 0 = predominantly one; 1 = two. (2)
22. Leaf type A glandular trichome length (mm). (1)
23. Leaf type A glandular trichome maximum width (mm). (1)
24. Type A glandular trichomes, terminal cell shape: 0 = oval or oblong-oval; 1 = oblong. (2)
25. Type A glandular trichomes, terminal cell length (mm). (1)
26. Type B glandular trichomes on leaf margins and adaxial lamina surface: 0 = absent; 1 = present. (2)
27. Midrib raised on abaxial leaf surface: 0 = absent; 1 = present. (2)
28. Epidermis thickness: 0 = thicker on adaxial lamina surface; 1 = equal thickness on both lamina surfaces. (2)
29. Distribution of stomata on leaf: 0 = on both adaxial and abaxial surfaces; 1 = on abaxial surface only. (2)
30. Lamina structure: 0 = dorsiventral; 1 = equifacial. (2)
31. Mesophyll differentiation: 0 = well differentiated; 1 = moderately or poorly differentiated. (2)
32. Spongy chlorenchyma: 0 = absent; 1 = present. (2)

33. Maximum width of central chlorenchyma cells: 0 = up to 30 μm; 1 = 30–60 μm; 2 = up to 80 μm. (1)
34. Sclerenchyma on adaxial side of midrib: 0 = absent; 1 = narrower than or similar width to vascular bundle; 2 = broader than vascular bundle. (1)
35. Sclerenchyma on abaxial side of midrib and major lateral veins: 0 = absent; 1 = present. (2)
36. Idioblastic sclereids in petiole: 0 = absent; 1 = present. (2)
37. Number of leaf traces: 0 = one only; 1 = one to three; 2 = three only. (1)
38. Morphologically distinct flowering shoots: 0 = absent; 1 = present. (2)
39. Transition from leaves to involucral bracts: 0 = abrupt; 1 = gradual. (2)
40. Length of leaves subtending capitulum: 0 = similar to leaves on nonflowering shoots; 1 = intermediate between leaves and involucral bracts; 2 = similar to involucral bracts; 3 = longer than involucral bracts. (1)
41. Morphology of leaves subtending the capitulum: 0 = similar to leaves on nonflowering shoots; 1 = intermediate between leaves and involucral bracts; 2 = morphologically distinct from leaves and involucral bracts. (2)
42. Indumentum on leaves subtending the capitulum: 0 = clothing trichomes project further beyond apex than in leaves on nonflowering shoots; 1 = clothing trichomes similar to leaves on nonflowering shoots or only extend laterally. (2)
43. Capitulum pedunculate: 0 = absent; 1 = present. (2)
44. Number of capitula per inflorescence. (1)
45. Capitulum length (mm). (1)
46. Capitulum width at midpoint (mm). (1)
47. Number of female florets per capitulum. (1)
48. Number of hermaphrodite florets per capitulum. (1)
49. Total number of florets per capitulum. (1)
50. Female: hermaphrodite floret ratio. (1)
51. Receptacle diameter (mm). (1)
52. Receptacle height (mm). (1)
53. Receptacle type: 0 = fimbrillate; 1 = foveolate; 2 = scrobiculate. (2)
54. Receptacle shape: 0 = conical; 1 = flat to convex. (2)
55. Inner involucral bract length (mm). (1)
56. Inner involucral bract, maximum width (mm). (1)
57. Colour of lamina of inner involucral bracts: 0 = white; 1 = pale brown; 2 = blackish-brown. (2)
58. Lamina of inner involucral bracts hygroscopic: 0 = absent; 1 = present. (2)
59. Shape of lamina apex of inner involucral bracts: 0 = acute; 1 = acute to obtuse; 2 = obtuse to rounded. (1)
60. Coloration in lamina/stereome gap of outer involucral bracts: 0 = translucent; 1 = pale brown; 2 = mid-brown. (1)
61. Hyaline margins on stereome of inner involucral bract: 0 = 75–125 μm; 1 = 125–200 μm; 2 = 200–325 μm. (1)
62. Corolla tube length in female florets (mm). (1)
63. Corolla tube length in hermaphrodite florets (mm). (1)
64. Hermaphrodite floret corolla tube, width at base of lobes (mm). (1)
65. Hermaphrodite floret corolla tube, point of expansion from base:total length. (1)
66. Lower corolla tube crimson at anthesis: 0 = absent; 1 = present. (2)
67. Corolla lobe and upper corolla tube colour at anthesis: 0 = pale green; 1 = white; 2 = crimson; 3 = yellow. (2)
68. Corolla lobe apex colouration: 0 = translucent; 1 = pale green; 2 = white; 3 = yellow. (2)
69. Corolla lobe curvature: 0 = erect only; 1 = at least some lobes partly patent or recurved. (2)
70. Crimson coloration in anthers: 0 = absent; 1 = pale reddish; 2 = dark crimson. (1)
71. Style colour: 0 = white; 1 = pale green; 2 = pale greenish-yellow; 3 = yellow. (2)
72. Style arm length in hermaphrodite florets (mm). (1)
73. Pappus hair length in female florets (mm). (1)
74. Pappus hair length in hermaphrodite florets (mm). (1)
75. Female-floret pappus hairs, number of apical cells. (1)
76. Hermaphrodite-floret pappus hairs, number of apical cells. (1)

77. Pappus hairs dimorphic between female and hermaphrodite florets: 0 = absent; 1 = present. (2)
78. Female-floret pappus hairs, apical cells distinctly protruding: 0 = absent; 1 = present. (2)
79. Hermaphrodite-floret pappus hairs, apical cells distinctly protruding: 0 = absent; 1 = present. (2)
80. Female-floret pappus hairs, shape of apical cells: 0 = acute; 1 = obtuse; 2 = clavate. (2)
81. Hermaphrodite-floret pappus hairs, shape of apical cells: 0 = obtuse; 1 = clavate. (2)
82. Pappus hair distinctly flattened and broader below apex: 0 = absent; 1 = present. (2)
83. Type of wall thickening in pappus-hair apical cells: 0 = reticulate or irregular; 1 = uniformly thickened. (2)
84. Length of basal spines on pappus hairs: 0 = up to 15 μm long; 1 = up to 30 μm long; 2 = up to 100 μm long. (1)
85. Angle of basal spines on pappus hairs: 0 = ascending only; 1 = ascending or spreading; 2 = ascending, spreading or recurved. (1)
86. Pappus hair basal spine apex shape: 0 = acute in at least some spines; 1 = obtuse. (2)
87. Female-floret ovary length (mm). (1)
88. Female-floret ovary width (mm). (1)
89. Female-floret ovary length: width ratio. (1)
90. Hermaphrodite-floret ovary length (mm).(1)
91. Hermaphrodite-floret ovary width (mm). (1)
92. Hermaphrodite-floret ovary length:width ratio. (1)
93. Ovary epidermis: 0 = cells rounded; 1 = cells not rounded. (2)
94. Twin hairs on ovary of both female and hermaphrodite florets: 0 = absent; 1 = present. (2)
95. Ovary twin hair length (mm). (1)
96. Ovary twin hairs, shape of terminal cells: 0 = acute; 1 = obtuse. (2)
97. Ovary twin hairs, fusion of terminal cells: 0 = coherent to the apex; 1 = free at the apex. (2)
98. Glandular trichomes on ovary of both female and hermaphrodite florets: 0 = absent; 1 = present. (2)

5.2 Characters included in each analysis

Key: †, log transformed; ‡, square-root transformed.

Character	All sympatric species included		Only putative hybrids and likely parental species included		
	Clustering, MDS, Split decomposition	MDA	Character count	Character index	Clustering, MDS, Split decomposition
1	✓		✓	✓	✓
2	✓				
3	✓				
4	✓	✓†	✓	✓	✓
5	✓	✓†	✓	✓	✓
6		✓†			
7	✓				✓
8	✓		✓	✓	✓
9	✓				
10	✓	✓†	✓	✓	✓
11	✓				
12	✓				
13	✓		✓	✓	✓
14	✓		✓	✓	✓
15	✓		✓	✓	✓
16	✓		✓	✓	✓
17	✓			✓	✓
18	✓	✓†			✓
19	✓	✓	✓	✓	✓
20	✓	✓	✓	✓	✓
21	✓				
22	✓	✓†			✓
23	✓	✓			✓
24	✓				
25	✓	✓			✓
26	✓				
27	✓				
28	✓				
29	✓				
30	✓				
31	✓		✓	✓	✓
32	✓				
33	✓		✓	✓	✓

5.2 Characters included in each analysis (continued)

Character	All sympatric species included		Only putative hybrids and likely parental species included		
	Clustering, MDS, Split decomposition	MDA	Character count	Character index	Clustering, MDS, Split decomposition
34	✓		✓	✓	✓
35	✓				✓
36	✓		✓	✓	✓
37	✓		✓	✓	✓
38	✓		✓	✓	✓
39	✓		✓	✓	✓
40	✓		✓	✓	✓
41	✓		✓	✓	✓
42	✓		✓	✓	✓
43	✓		✓		
44	✓	✓†	✓	✓	✓
45	✓	✓	✓	✓	✓
46	✓	✓†	✓	✓	✓
47	✓	✓‡	✓	✓	✓
48	✓	✓‡	✓	✓	✓
49		✓‡	✓	✓	
50		✓		✓	
51	✓	✓†	✓	✓	✓
52	✓	✓†	✓	✓	✓
53	✓			✓	
54	✓				
55	✓	✓	✓	✓	✓
56	✓	✓†	✓	✓	✓
57	✓		✓	✓	✓
58	✓				
59	✓		✓	✓	✓
60	✓		✓	✓	✓
61	✓		✓	✓	✓
62	✓	✓	✓	✓	✓
63	✓	✓†			✓
64	✓	✓	✓	✓	✓
65	✓	✓‡		✓	✓
66	✓			✓	✓
67	✓		✓	✓	✓
68	✓		✓	✓	✓
69	✓				✓
70	✓		✓	✓	✓

5.2 Characters included in each analysis (continued)

Character	All sympatric species included		Only putative hybrids and likely parental species included		
	Clustering, MDS, Split decomposition	MDA	Character count	Character index	Clustering, MDS, Split decomposition
71	✓		✓	✓	✓
72	✓	✓†			✓
73	✓	✓†		✓	✓
74	✓	✓†			✓
75	✓	✓			✓
76	✓	✓‡			✓
77	✓				
78	✓			✓	
79	✓				
80	✓		✓	✓	✓
81	✓				✓
82	✓				
83	✓				✓
84	✓		✓	✓	✓
85	✓		✓	✓	✓
86	✓				
87	✓	✓			✓
88	✓	✓†		✓	✓
89		✓			
90	✓	✓		✓	✓
91	✓	✓†			✓
92			✓†		
93	✓				
94	✓				
95	✓	✓†	✓	✓	✓
96	✓				
97	✓				
98	✓				